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# **PROCEEDINGS**

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PART IV

REVISION OF BRACHYLAEMIDAL JOYEUX ET FOLEY, 1930 WITH NEW SUBFAMILIES THAPARIELLINAE AND UROTREMATINAE AND

NEW FAMILY HARMOTREMATIDAE WITH ITS SUBFAMILIES, HARMOTREMATINAE YAMAGUTI, 1933 AND HELICOTREMATINAE N. SUBF.

By
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Allakabad

[Received on 20th February, 1962]

The families Thapariellidae Srivastava, 1953 and Urotrematidae Poche, 1926 are dropped. They are reduced to the rank of subfamilies Thapariellinae n. subf. and Urotrematinae respectively and included in the family Brachylaemidae Joyeux et Foley, 1930. The family Leucochloridiidae Dollfus, 1934 is recognised as subfamily Leucochloridiinae Poche, 1907 as was done by us (1936). Allison (1943), Dawes (1956) and Kagan (1952) have treated it as a subfamily. Yamaguti (1958) has split up Brachylaemidae into families, Hasstilesiidae Hall, 1916, Leucochloridiidae Dollfus, 1934, Liolopidae Dollfus, 1934 and Moreauiidae Yamaguti, 1958. The subfamilies Liolopinae Odhner, 1912, parasitic in Amphibia, Harmotrematinae Yamaguti, 1933 parasitic in reptiles and Moreauiinae Johnston, 1915 parasitic in Monotremata treated previously by us (1936) should be elevated to the rank of families within the superfamily Brachylaemoidea (suborder Brachylaemata of order Strigeatoidea). The families Liolopidae Dollfus, 1934 and Moreauiidae Yamaguti, 1958 are accordingly accepted, We create the family Harmotrematidae n. fam. dividing it into two subfamilies Harmotrematinae Yamaguti, 1933 and Helicotrematinae n. subf. The Harmotrematidae n. fam., parasitic in reptiles is distinguished from the Liolopidae Dollfus, 1934 by the absence of vesicula seminalis externa, presence of large cirrus sac, ventrolateral sinistral position of genital pore, and small uterus containing a few or moderate number of large thick shelled ova. The receptaculum seminis is present. Two pairs of longitudinal exretory trunks extend along whole length of body. The Liolopidae, parasitic in Amphibians possesses vesicula seminalis externa, proportionately small cirrus sac and lacks prostatic complex and receptaculum seminis. The genital pore is ventral and submedian in position. The testes are separated by ovary and winding uterine coils,

and the uterus is moderately large containing numerous small and thin shelled ova. Thus Harmotrematidae n. fam. though related to the Liolopidae and assigned to the same superfamily Brachylaemoidea Allison is well differentiated as a family.

Harmotrematidae n. fam. contains two subfamilies Harmotrematinae Yamaguti, 1933 and Helicotrematinae n. subf. Harmotrematinae: Body spatulate or linguiform with narrow forebody and wider postacetabular and postequatorial hind body. Oral sucker very small, oesophagus short. Acetabulum larger than oral sucker and widely separated from anterior extremity. Testes entirely or largely in posterior half of body. Cirrus sac large, lying transversely or obliquely across entire breadth of intercaecal field; vesicula seminalis bipartite. Eggs a few only. Intestinal parasites of snakes and crocodiles.

Type genus: Harmotrema Nicoll, 1914.

Helicotrematinae n. subf.: Body long, slender, uniform in breadth. Acetabulum subequal to oral sucker and near anterior extremity. Pharynx comparatively large; oesophagus very short. Testes and ovary entirely in anterior half of body. Cirrus sac curved, postacetabular, pre-equatorial and vesicula seminalis winding. Eggs moderate in number. Intestinal parasites of Lacertae.

Type genus: Helicotrema Odhner, 1912.

The family Hasstilesiidae Hall, 1916 accepted recently by Yamaguti (1953) is dropped by us. We maintain it as subfamily Hasstilesiinae Hall, 1916 under the family Brachylaemidae Joyeux and Foley, 1930. The subfamily Hasstilesiinae resembles closely the subfamilies Brachylaeminae Joyeux and Foley, 1930 and Leucochloridiomorphinae Yamaguti, 1958 in the position of genital pore in testicular region and in the position and arrangement of gonads. In our opinion there does not seem to be a case for maintaining it as a family.

The family Leucochloridiidae Dollfus, 1934 also accepted by Yamaguti (1958) as already mentioned is considered as subfamily Leucochloridiinae Poche, 1907, in which we include only the single genus Leucochloridium Carus, 1835 syn. Urogonimus Monticelli, 1888, syn. Neoleucochloridium Kagan, 1951. We agree with Yamaguti (1958) in excluding Urorygma Braun, 1901 and Urotocus Looss, 1899 from this subfamily and creating subfamilies Urorygminae and Urotocinae respectively for these genera. The former resembles Brachylaeminae in the tandem position of testes, intertesticular ovary and intercaecal uterus, but it differs on account of the terminal position of genital pore. It has been included in Leucochloridiidae by Yamaguti on account of its terminal genital pore and habitat in birds. The subfamily Urorygminae Yamaguti is well differentiated from the subfamily Leucochloridiinae on account of large size of its suckers, acetabulum being much larger and the position of ovary dorsal to acetabulum quite separated from testes, the latter situated close to one another in forebody.

The family Urotrematidae Poche, 1926 is reduced to the rank of subfamily Urotrematinae n. subf. as it is closely related to Leucochloridiinae Poche, Urotocinae Yamaguti and Urorygminae Yamaguti which it resembles in the terminal position of genital pore and habitat of its genera in mammalian and reptilian hosts. The ovary is immediately postacetabular. The testes lie tandem, separated from ovary, in posterior half of body. The vitellaria are lateral and usually postacetabular. The excretory vesicle is V-shaped resembling that in Thapariellinae n. subf. The subfamilies Leucochloridiinae, Urorygminae, Urotocinae, Urotrematinae n. subf., Brachylaeminae, Hasstilesiinae, Scaphiostominae, Leucochloridiomorphinae and Thapariellinae n. subf. stand differing in some of their characters in a grading manner in such a way that they appear to have been evolved along

some divergent lines. The subfamilies Leucochloridiinae and Thapariellinae n. subf. have oval or somewhat elongated body, well developed suckers and dorsoterminal genital pore. A very short oesophagus is present in the former, but absent in the latter. Thapariellinae differs so remarkably in the small post-testicular vitellaria and uterus situated near posterior end and absence of cirrus sac from all the Brachylaemidae that it deserves the rank of a subfamily within the latter family Its so called excretory vesicle is V-shaped which resembles the short or reduced tubular stem with long cornua or arms of V or U of Brachylaemidae. In the Leucochloridiinae the vitellaria and uterus are extensively developed reaching in front of acetabulum to caecal arch or even further in front to pharynx or oral sucker. The excretory vesicle is vesicular or short tubular. In Leucochloridiomorphinae the excretory vesicule is similar, the suckers are well developed, the acetabulum much larger than oral sucker, the uterus and vitellaria are anterolateral to acetabulum. The position of genital pore midventral in testicular region, distinguishes this subfamily from Leucochloridiinae and Thapariellinae. The subfamilies Leucochloridiinae Poche, Thapariellinae n. subf. and Leucochloridiomorphinae Yamaguti are parasitic in birds; the first and the last are remarkably similar in being parasitic in bursa Fabricii.

Srivastava (1953) mentioned the features of resemblance of his genus Thapariella with Leucochloridium and Leucochloridiomorphia. Agarwal (1958) pointed out the close affinity of Thapariella anastemosa Srivastava, 1953 to the subfamily Leucochloridiinae due to its encystment in a snail host, its final host being a bird, in having genital pore at posterior end of body and having pars prostatica free in the parenchyma. We think that the snail tissue in the mouth cavity of the bird from which he obtained two cysts belonged to a terrestrial snail. He mentioned that the significant characters in which Thapariella differs from Leucochloridiinae are the post-testicular position of the ovary, post-testicular vitellaria and uterus which lie near hinder end and absence of cirrus sac. The cirrus sac is small in some species of Leucochloridium Carus, 1835. In Thapariella it has disappeared. The cirrus sac is absent in Postharmostomum helicis. The ovary is post-testicular in Leucochloridium cercatum (Mont.). Thapariella Srivastava, 1953 obviously belongs to a new subfamily under the Brachylaemidae. In the genus Postharmostomum (Brachylaeminae) the cirrus sac is present or absent. The so called V-shaped exretory vesicle of Thapariella represents really the narrow long cornua or siphons, the main stem being reduced or lacking. I am of opinion that it is similar to that of Brach-

laemidae.

The position of genital pore in Leucochloridium perisorisae Neuland, 1953 as shown in the figure is not terminal. It lies a short distance in front of blind ends of caeca and posterior extremity. It seems that its position in the figure is not correctly shown. The author, hewever, mentioned that the figure was drawn with the help of camera lucida and the number of specimens obtained was 27. If the position of the genital pore is correctly shown in the figure, this species may come under a new genus as the genital pore shows a great departure from its terminal position in the genus. The author is, however, silent in the description of the species about the genital pore.

The genera Neoleucochloridium Kagan, 1951, and Urogonimus Monticelli, 1888 (revived by Kagan, 1951) are not considered tenable as they cannot be clearly differentiated from Leucochloridium Carus, 1835. The species of Neoleucochloridium have intracaecal uterus, long smooth, pustulated cirrus, Laurer's canal long opening in the excretory bladder, testes and ovary in a triangular position, the vitellaria extracaecal and host birds belong to the family Rallidae. All the characters are variable. Kagan (1952) himself mentions "Species of Neoleucochloridium gen, nov. in certain respects are very similar to species of Leucochloridium Carus,

1835". Testes and ovary are in triangular position in both Leucochloridium and Neoleucochloridium. The Laurer's canal opens in excretory bladder in Leucoc' loridium, also. In Urogonimus Monticelli it opens to the exterior on the dorsal surface of the body. Cirrus in the latter is short and stubby lying in the genital atrium and not in cirrus sac; pars musculosa thick and bulbous; genital glands are in a straight line in Urogonimus. Yamaguti (1939) described two new species L. turdi and L. cardis. L. cardis which Yamaguti considers closely related to L. seiuri McIntosh, 1933 differs from the latter species in the size of suckers and the ovary and testes in not being arranged in a linear series, but in the figure of the species he shows the arrangement of gonads almost in a straight line, the ovary slightly projecting out close towards the caecum of that side. The Laurer's canal opens on the mid-dorsal surface at the level of posterior testis in this species. Uterus in L. cardis though largely intracaecal extends over the caeca at places and vitellaria extend much in front of acetabulum, reaching anterior to pharynx. So this species can neither be assigned to Neoleucochloridium, nor to Urogonimus. It combines the characters of latter genus and Leucochloridium. L. turdi Yamaguti, 1339 has its uterus extending laterally over caeca and extensive vitellaria like L. cardis. Its pars musculosa is broader than tubular pars prostatica, and cirrus sac is muscular containing long twisted cirrus. This species can also be assigned to Leucochloridium. L. fuscostriatum Robinson, 1947 has no part of the uterus lateral to caeca and the cirrus sac contains long, coiled cirrus. Its vitellaria are extensive and extra-caecal extending to oral sucker. The gonads are not in a straight line; the ovary lies just in front of and in line with posterior testis, whereas anterior testis lies just behind acetabulum. This species, which stands close to L. cyanocittae, L. variae and L. actitis cannot be assigned to Neoleucochloridium. The position of external opening of Laurer's canal is not mentioned. We think that the condition of uterus extending outside caeca cannot be considered of generic value as the extension of uterus whether outside or inside caeca is subject to much variation. The position of external opening of Laurer's canal has not been determined by many authors and therefore has not much systematic value ordinarily. The cirrus whether long, pointed or fusculated does not seem to be of much significance. The character of host specificity whether in marsh birds Rallidae or shore or passerine birds is not of reliable nature. The genus Neoleucochloridium, therefore cannot be maintained. Urogonimus Monticelli, which was dropped by many authors cannot be differentiated from Leucochloricium Carus. We agree with McIntosh that the distribution of vitellaria and uterus and arrangement of gonads are subject to variation in different individuals of a species. Kagan, however, considers them as valid for generic and specific determination.

Neiland (1953) mentions that in *L. periscrisae* Neiland the ovary which is slightly medial to posterior testis in one specimen is directly in line with both the testes. This shows that the gonads are very variable from their arrangement in a triangle to that in a linear series. This species, which possesses armed cirrus has been assigned to *Leucochloridium*. The vitellaria are ventral and overlap the caeca, but they are not completely lateral to them. We have already mentioned that the position of the genital pore, which lies anterior to blind ends of caeca and thus is not terminal, gives it a unique position in the genus. The position of the genital pore should be re-examined in the entire mounts of this species.

The colour and pattern of brood sac cannot be taken into account ordinarily as a generic character. Robinson (1947) says that it is not certain to what extent the colour and pattern of the sporocyst are dependable in determining species of Leucochloridium and in this view he is supported by Wesenberg Lund (1931) and Lutz (1921), Woodhead (1935) and Hsu (1936).

## Remarks on the phylogeny of the subfamilies of Brachylaemidae

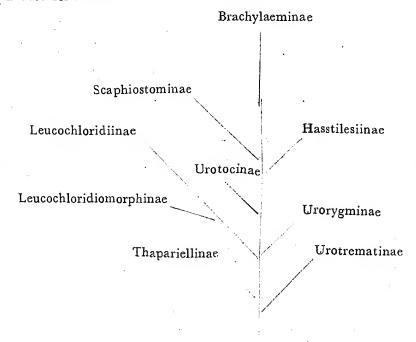
The ancestor of the family possessed post-acetabular genital pore in testicular zone or near it in front of the posterior extremity and was probably parasitic in Reptilia. From it two lines diverged off—one giving the subfamily Brachylaeminae, Scaphistominae parasitic in birds and mammals and Hasstilesiinae parasitic in mammals and the other branch resulted in the Urotrematinae n. subf.

The position of Urotrematidae Poche, 1926 which we have reduced to a subfamily has been a difficult question. Poche (1925) created Urotrematidae to accommodate Urotrema Braun, 1900. Viana (1924) had included the genus in Harmostomidae without giving any reasons. Poche thinks that as it possesses a V-shaped excretory vesicle, a large cirrus sac containing vesicula seminalis, and ovary situated far in front and separated from testes, lacking ascending part of uterus and a long oesophagus present, it cannot come under Harmostomidae according to the diagnosis given by Odhner in 19'2. He says that Harmostomidae on the contrary have Y-shaped excretory vesicle with a very short stem, small oesophagus, small cirrus sac, vesicula seminalis lying freely in parenchyma, ovary situated between testes and with ascending uterine coils. The excretory vesicle is short tubular or vesicular with long tubes or siphons in Brachylaemidae. It is not Y-shaped in the usual sense. In Thapariellinae n. subf., which clearly belongs to the Brachylaemidae, the excretory vesicle is V-shaped. The oesophagus is usually short in the family and cirrus sac large in some Brachylaemidae. In the Brachylaemidae vesicula seminalis and prostatic complex may be outside or within cirrus sac. The ovary is separated from the testes and lies dorsal to acetabulum in Urorygma Braun (subfamily Urorygminae Yamaguti, 1958). The entirely descending uterus in *Urotrema* Braun is due to anterior post-acetabular position of ovary, so this character alone should not determine for the genera *Urotrema* Braun, 1900 and Urotrematulum Macy, 1933 a place in a separate family. The character of host specificity of the genera mostly parasitic in Chiroptera is, however, a point to permit their exclusion from Brachylaemidae. But the position of the genital pore at or near the posterior extremity is decisively in favour of including them in Brachylaemidae, till the life cycle when known demonstrates to the contrary against this. We, therefore, reduce the Urotrematidae to Urotrematinae n. subf. and include it in the Brachylaemidae.

It is well known that vesicula seminalis may lie within cirrus sac or outside it as in Leucochloridiinae and Leucochloridiomorphinae. In Thapariellinae n. subf. the cirrus sac is absent. In Postharmostomum helicis (Brachylaeminae) the cirrus sac is absent (Ulmer, 1951). The cirrus sac has thus disapeared in some genera of Brachylaemidae. The Urotrematinae were separated off quite early as a branch from the ancestor of the family Brachylaemidae. The subfamily Leucochloridiomorphinae containing the genus Leucochloridiomorpha Gower, 1938 occupies a position near the origin of the main Brachylaemid branch as a starting point for the subfamily Leucochloridiinae, Poche, 1907. Both these subfamilies are parasitic in birds and also in bursa Fabricii. The Leucochloridiomorphinae are primitive representing a connecting link with the ancestor of the family for they have a free swimming furcocercous larva developing in aquatic snails. The tail-less cercariaeum of Leucochloridium (Leucochloridinae) has been evolved separately from that of Brachylaeminae, as the cercaria of Postharmostomum gallinum (Witeberg, 1925) possesses a short vestigial tail "square with excretory pore at the corners of the tail". Postharmostomum helicis (Brachylaeminae) as already pointed out lacks a cirrus pouch (Ulmer, 1951). No vestige of tail occurs in cercaria of Leucochloridium. Leucochloridiinae has thus been evolved separately from Brachylaeminae as an

adaptation to its development in terrestrial snails from the other Brachylaemidae. The subfamily Thapariellinae n. subf. arose near the origin of the Leucochloridiinae branch as an isolated twig possessing post testicular vitellaria and uterus and lacking a cirrus sac. This subfamily stands close to the Leucochloridiinae on account of its dorsoterminal genital pore, presence of vesicula seminalis externa and pars prostatica. The cirrus sac is absent in this subfamily. It is also parasitic in birds.

The Urotocinae arose as a side branch at the base of the subfamily Brachy-laeminae for forms with terminal genital pore. The testes and ovary are similar in shape and position to those in the Brachylaeminae. The Urorygminae containing the specialized aberrant genus Urorygma Braun, 1901 with terminal genital pore and large suckers and peculiar position of gonads arose as a branch between the Leucochloridinae and Urotocinae branches.



Ancestor of Brachylaemidae

Brachylaemidae Joyeux et Foley, 1930. syn. Harmostomidae Odhner, 1912.

Family diagnosis.—Distomes of varying forms, elongated, oval, sole-shaped, subglobular, ribbon-shaped or slender, long and filiform. Size small or medium. Cuticle smooth or spinate. Acetabulum large or small, near oral sucker, or subequatorial, equatorial or immediately postequatorial. Genital pore post-acetabular, median, submedian or lateral, ventral near posterior extremity, terminal or dorso-terminal, or ventral a little distance further in front near testes or testicular zone. Gonads more or loss in the same line or in a triangular arrangement, postacetabular, postequatorial, testes occasionally in forebody and ovary dorsal, to acetabulum (Urorygminae). Testes two, usually intercaecal, tandem or oblique

near posterior extremity exceptionally preacetabular in forebody (Urorygminae). Cirrus sac usually small, vesicular anterolateral or ventral to anterior testis or anterior or behind posterior testis near hinder end of body, sometimes small containing only cirrus, sometimes containing seminal vesicle and prostate complex. Cirrus sac absent in a few Brachylaeminae and Thapariellinae n. subf. Vesicula seminalis and prostate complex outside or inside cirrus sac. Ovary intertesticular or lateral or variable in position with respect to testes. Vitellaria follicular lateral usually stripe like, variable in extent to hinder end of overy or end of body or confined only to post testicular region (Thapariellinae). Uterus coiled, intercaecal or overlapping caeca anterior to gonads reaching to intestinal bifurcation or even or al sucker or pharynx, exceptionally confined to post-testicular region (Thapariellinae). Metraterm usually present. Eggs small numerous. Excretory vesicle small U or V-shaped, saccular or tubular with a short narrow stem and two long narrow tubular cornua or siphons extending inside or outside caeca to oral sucker. Parasites of birds and mammals, sometimes reptiles. Location gut, cloaca or bursa Fabricii of birds. Free living miracidium absent as a rule. Cercaria exceptionaly furcocercous (Leucochloridiomorphinae), usually with much reduced tail or tail-less. Sporocysts simple or branched developing in snails, usually terrestrial snails, producing comparatively a few cercariae. Metacercariae in snails.

Type genus: Brachylaema Duj., 1843.

Key to subfamilies of Brachylaemidae.

ì.	Cirrus sac absent. Vitellaria and uterus confined to post-testicular region
	Cirrus sac present exceptionally absent in Brachylaeminae (Pisthamostomum). Vitellaria and uterus extensive not confined to post-testicular region
2.	Genital pore dorso-terminalLeucochloridinae Poche.
	Genital pure near testes or near testicular zone
3.	Ovary post-acetabular; suckers small, subequal; parasitic in mammals and reptiles
	Ovary dorsal to acetabulum; suckers large, acetabulum very large;
٠	Ovary median intertesticular; parasitic in birds
4.	Ovary lateral in testicular zone; acetabulum small; parasitic in mammals
	Ovary pretesticular; acetabulum very large; parastic in bulsa Fabrici of birdsLeucochloridiomorphinae.
	Ovary intertesticular; body elongated long and filliorm
	Ovary intertesticular or opposite anterior testis; body elongated cylindrical or sole-shaped but not filiform Brachylaeminae Joyeux et Foley.

# syn. Harmostominae Braun, 1899; Heterolopinae Looss, 1899; Panopistinae Yamaguti, 1958.

Subfamily diagnosis.—Brachylaemidae: Body elongated, subcylindrical, linguiform, ribbonshaped, oblong or narrow, but not filiform. Cuticle spinulate or unspinulate. Acetabulum well developed, usually pre equatorial sometime equatorial. Oesophagus short, very short or absent; caeca straight or undulating. Genital pore ventral, median or submedian in testicular zone, a little in front of posterior end. Gonads in straight line or triangular position. Testes tandem or oblique in posterior third or fourth part of body. Ovary intertesticular. Cirrus sac small or moderate sized, vesicular containing cirrus only or vesicula seminalis prostate complex and cirrus, or cirrus sac sometime absent. Vesicula seminalis externa present or absent. Cirrus usually unarmed. Vitellaria usually stripe like and variable in length. Uterus large coiled, intracaecal, sometime overapping caeca laterally. Excretory vesicle tubular, short with a short stem and long cornua extending to oral sucker. Eggs small, numerous. Sporocysts in therestrial snails; tail of cercaria short, vestigial. Parasitic in birds and mammals.

Type genus: Brachylaema Dujardin, 1843.

Other genera are Glaplyrostomum Braun, 1901, Postharmostomum Witenberg, 1923, Ectosiphonus Sinitzin, 1931, Entosiphonus Sinitzin, 1931 and Panopistus Sinitzin 1931.

Key to genera of subfamily Brachylaeminae
Genital pore near posterior extremity; cirrus sac post-testicular
Genital pore in testicular zone or just pretesticular
1. Caeca strongly winding or serpentine Postharmostomum Witenberg, 1923.  Caeca straight or somewhat sinuous
2. Acetabulum nearer anterior end than to middle of body; uterus not extending anterior to acetabulum; prepharynx muscular resembling pharynx
Acetabulum nearer middle of body than to anterior end3
3. Excretory bladder extracaecal. Vitellaria extending in posterior half of body. Genital pore in front of anterior testis. Prepharynx tubular, flexible but not muscular; parasitic in mammals
Excretory bladder intracaecal. Vitellaría extending from acetabular zone or a little in front4
4. Genital pore intertesticular; uterine coils reaching to acetabulum or to pharynx; parasitic in birds
Genital pore pre- or intertesticular; uterus turning back on itself behind intestinal bifurcation; parasitic in birds or mammals
Scaphistominae Yamaguti, 1958

Scaphistominae Yamaguti, 1958 syn. Ityogoniminae Yamaguti, 1958

Subfamily diagnosis.—Brachylaemidae: Body narrow, filiform and long sucker well developed. Prepharynx and oesophagus very short; caeca termi

nating at or near hinder end. Acetabulum near or far apart from anterior end. Testes tandem in posterior third or fourth part of body near one another closely separated by ovary or wide apart. Cirrus sac pretesticular or in front of posterior testis; the latter situated near hinder end. Genital pore in front of anterior testis or posterior testis. Ovary intertesticular. Uterus intercaecal, occupying greater part of hind body reaching acetabulum or ending some distance behind it. Vitellaria extending from behind acetabulum to anterior testis or ovary. Parasitic in birds and mammals.

Type genus: Scaphistomum Braun, 1901. Other genus: Ityogonimus Luhe, 1899.

Key to identification of genera of Scaphistominae Yamaguti, 1958.

## Hasstilesiinae Hall, 1916

Subfamily diagnosis.—Brachylaemidae: Body small, oval, spinate. Suckers small, nearly equal. Pharynx small; oesophagus very short; ceaca more or less sinuous terminating at or near hinder end. Genital pore ventral submedian or lateral, submarginal postequatorial or almost half way between acetabulum and hinder end. Gonads arranged in a triangle. Testes oblique, post-equatorial near hinder end. Cirrus sac relatively large, flask-shaped, pyriform or sigmoid, submedian, intertesticular or in front of testes. Vesicula seminalis outside cirrus sac. Cirrus unarmed. Ovary ventral to right caecum, lateral to posterior testis or in front of it. Receptaculum seminis absent. Uterus massed laterally in anterior region or intercaecal between testes and intestinal bifurcation. Eggs small, numerous. Parasitic in intestine of mammals (rabbits and ruminants)

Type genus: Hasstilesia Hall, 1916.

Other genus : Skrjabinotrema Orlaff, Erschoff et Badanin, 1934.

# Leucochloridiomorphinae Yamaguti, 1958

Subfamily diagnosis.—Brachylaemidae: Body somewhat elongated oval. Suckers well developed; acetabulum much larger, enormous, just post-equatorial. Genital pore mid-ventral in testicular region. Gonads post-acetabular arranged in triangle with ovary anterior. Testes justaposed at posterior end. Cirrus sac enclosing cirrus only. Cirrus armed. Vesicula seminalis outside cirrus sac. Ovary pretesticular. Vitellaria lateral, short, in anterior half of body behind intestinal bifurcation. Uterus with ascending and descending limbs on each side of acetabulum. Eggs small, numerous. Cercaria furcocercous with natatory tail developing in branched sporocyst in aquatic snails. Parasitic in bursa Fabricii of birds.

Type genus: Leucochloridiomorpha Gower, 1938.

Genotype and only species: Leucochloridiomorpha constantiae (Mueller, 1935).

# Urotocinae Yamaguti, 1958.

Subfamily diagnosis.—Brachylaemidae: Body elongated, narrow, tongue-shaped. Suckers weakly developed; acetabulum very small, pre-equatorial nearer to middle of body. Prepharynx absent. Pharynx small. Oesophagus absent, caeca extending to near hinder end. Genital pore terminal. Gonads in straight line. Testes tandem near hinder end. Cirrus sac small, post-testicular. Ovary intertesticular. Vitellaria lateral, stripe-like, extending from a little in front of acetabulum to ovary or anterior testis leaving free two extremities of body. Uterus coiled, intercaecal, between caecal arch and anterior testis. Eggs small, numerous. Parasitic in intestine and bursa Fabricii of birds.

Type genus: Urotocus Looss, 1899.

## Leucochloridiinae Poche, 1907

Subfamily diagnosis.—Brachylaemidae: Body oval or elongated oval. Cuticle smooth or spinate. Suckers strongly developed, large. Acetabulum subequatorial, equatorial or immediately postequatorial. Pharynx well developed, but small relatively to oral sucker. Oesophagus very short almost absent. Caeca simple terminating near or at posterior extremity. Genital pore dorso-terminal. Gonads in hind body in straight line or in triangular arrangement. Testes tandem or diagonal. Cirrus sac at extreme posterior end of body. Ovary intertesticular or opposite anterior testis or immediately in front of posterior testis. Receptaculum seminis absent or formed by dilatation of Laurer's canal. Vitellaria lateral extending in fore and hind body approaching caecal arch, or extending to pharynx and oral sucker. Uterus large, coiled, ascending and descending, extending from in front of acetabulum near caecal arch to near hind end. Ova small, numerous. Excretory vesicle small, saccular or tubular. Cercariae completely tail-less (cercariaeum) developing in terrestrial snails in sporocysts and move into special modifications, brood sac or special branch of sporocyst where they develop into infective metacercariae. Parasitic in cloaca and bursa Fabricii of birds.

Type genus: Leucochloridium Carus, 1845. syn. Urogonimus Monticelli, 1888, and Neoleucochloridium Kagan, 1951.

# Urorygminae Yamaguti, 1958

Subfamily diagnosis.—Brachylaemidae: Body small, elongate oval, unspined. Suckers very large. Acetabulum very large, post equatorial. Prepharynx absent. Pharynx large. Oesophagus absent; caeca reaching hinder end. Genital pore terminal. Testes juxtaposed, preacetabular between intestinal bifurcation and acetabulum. Ovary submedian dorsal to acetabulum. Receptaculum seminis absent. Vitellaria in lateral fields preacetabular, pre-equatorial. Uterine coils over-reaching caeca laterally. Eggs small, numerous. Parasitic in birds.

Type genus: Urorygma Braun, 1901.

Genotype and only species: Urorygma nanodes Braun, 1901.

# Thapariellinae n. subf.

Subfamily diagnosis.—Brachylaemidae: Body somewhat elongate oval with rounded ends. Suckers well developed. Acetabulum pre-equatorial, larger than oral sucker. Prepharynx absent; pharynx well developed. Oesophagus absent; caeca narrow and undulating, reaching a little in front of hinder end. Genital pore dorso-terminal. All gonads confined to posterior third of body, Testes slightly oblique. Cirrus sac absent. Vas deferens winding, vesicula seminalis

present. Prostatic complex and pars musculosa well developed. Cirrus spined. Ovary post-testicular, submedian; shell gland complex immediately post-ovarian. Receptaculum seminis absent. Genital atrium surrounded by gland cells. Vitellaria follicular, lateral in ovarian zone, composed of a few 6-8 bunches of follicles, post-testicular. Uterus post testicular with ascending and descending limbs. Eggs small, numerous. Excretory vesicle lacking median stem, V-shaped; main excretory ducts run entire length of body and coil by side of pharynx and oral sucker. Parasitic in birds.

Type genus: Thapariella Srivastava, 1953.

Genotype and single species: Thapariella anastomusa Srivastava, 1953.

## Urotromatinae n. subf.

Subfamily diagnosis.—Brachylaemidae: Body small, elongated, fusiform, spinulate. Suckers and pharynx small. Acetabulum subequal to oral sucker, preequatorial. Prepharynx very short; oesophagus short or moderately long. Genital pore terminal or subterminal at caudal end. Testes tandem, intercaecal, post equatorial a little in front of hinder end. Cirrus sac present, pyriform containing vesicula seminalis. Ovary post-acetabular, pre-equatorial much in front of testes. Receptaculum seminis present. Vitellaria lateral postacetabular extending from level of acetabulum along caeca to anterior end of anterior testis. Uterus winding descending backward, consisting of descending limb only. Eggs small, numerous, thick shelled. Excretory vesicle V-shaped. Parasitic in mammals and reptiles.

Type genus: *Urotrema* Braun, 1900. Other genus: *Urotrematulum* Macy, 1933.

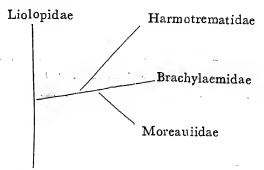
## Harmotrematidae n. fam

The systematic position of Harmotrema was reviewed by us (1936). The subfamily Liolopinae Odhner, 1912 was raised to the family Liolopidae by Dollfus (1934), who divided it into two subfamilies Liolopinae Odhner and Moreauiinae Johnston, 1915. To the former were assigned the genera Liolope Cohn, 1902, Harmotremo Nicoll, 1914 and Helicotrema Odhner, 1912. Yamaguti (1933) described Harmotrema laticaudae and created subfamily Harmotrematinae under Clinostomidae, but he thought that it did not fit well in this family and it possibly represents a new family. Dollfus (1934), however, considered it synonymous to his family Liolopidae. Yamaguti (1958) has accepted the latter family and divided it into the subfamilies Liolopinae Odhner and Harmotrematinae Yamaguti, including the amphibian genus Liolope, in the former and reptilian genera Harmotrema and Helicotrema in the latter. He has raised Moreauiinae to the rank of the family Moreauiidae Yamaguti, 1958. Allison (1943) suggested that the Liolopinae, Moreauiinae and Harmotrematinae should be raised to the rank of families.

Harmotrema Nicoll and Helicotrema Odhner show close affinities with Liolope Cohn, but the differences between them are wide enough to necessitate their inclusion in separate families, Harmotrematidae n. fam. and Liolopidae Dollfus. The voluminous vesicula seminalis, situated outside cirrus sac and in front of anterior testis in the latter in contrast to that in Harmotrematidae, where it lies within cirrus sac along with the prostatic complex and cirrus, is the essential difference. The pars prostatica is poorly developed or absent in Liolope, and the testes are separated from one another by the ovary and uterine coils in this genus. The uterus is much smaller in Harmotrematidae n. fam. containing a few large.

thick shelled ova, whereas in Liolopidae it is large containing many small thin shelled ova. These differences as well as the hosts belonging to different classes, Amphibia and Reptilia justify the elevation of Harmotrematinae to the level of a family. Moreover, Helicotrema differs so much from Harmotrema that the allocation of these genera to two separate subfamilies Helicotrematinae n. subf. and Harmotrematinae Yamaguti necessitate this grouping, so that Moreauiidae Yamaguti, parasitic in Monotremata, Harmotrematidae parasitic in Reptilia and Liolopidae parasitic in Amphibia are brought into line with one another showing their affinities and differences.

The Liolopidae is a primitive family, from which have been evolved Harmotrematidae, Moreauiidae and Brachylaemidae along divergent lines. Their evolution is closely bound up with the evolution of their hosts. The ancestor of the superfamily Brachylaemoidea which was parasitic in an amphibian host evolving into a reptile, forms a keynote of the parallel evolution of these parasites and their hosts. The Brachylaemidae became established in reptiles, birds and mammals, the Moreauiidae became confined to and isolated in Monotremes like their hosts, whereas the Liolopidae and Harmotrematidae have maintained their primitive habitat in their amphibian and reptilian hosts.



Ancestor of Brachylaemoidea

Diagnosis of Harmotrematidae n. fam.—Brachylaemoidea: Eody small or moderate sized, elongated, spatulate or linguiform, broad pasteriorly (Harmotrematinae); slender filiform of uniform breadth (Helicotrematinae). Cuticle spinate or with rudimentary spines. Suckers small. Acetabulum subequatorial or near anterior end. Prepharynx absent; pharynx present; oesophagus short, very short or absent; caeca long, narrow, simple reaching near hinder extremity. Genital pore postacetabular, pretesticular, pre-equatorial or postequatorial, ventrolateral, much sinistral, submarginal or ventral to left caecum. Testes close or apart from one another separated by ovary, shell gland complex in posterior or anterior half of body. Girrus sac postacetabular, pretesticular, large, curved lying transversely or obliquely across entire breadth of intercaecal field (Harmotrematinae) or to left side (Helicotrematinae). Vesicula seminalis bipartite or winding within cirrus sac. Prostatic complex well developed. Cirrus large and armed with spines. Vesicula seminalis externa absent. Ovary intertesticular. Receptaculum seminis present. Vitellaria follicular, extensively developed, in forebody and hind body along greater length of caeca separate or confluent posteriorly, more or less leaving anterior part of caeca free. Uterus short winding forward by side of or dorsal to anterior testis (Harmotrematinae) or moderately long, rectilinear from ovarian

complex to genital pore on left side '(Helicotrematinae). Metraterm well differentiated, spined. Eggs a few or moderate in number, large sized and thick shelled. Excretory system of short tubular vesicle with one or two pairs of long excretory vessels extending whole length of body, united anteriorly behind pharynx. Parasitic in intestine of Lacertae, snakes and crocodiles.

Type genus: Harmotrema Nicoll, 1914.

## Harmotrematinae Yamaguti, 1933

Subfamily diagnosis.—Harmotrematidae n. fam.: Body spatulate or linguiform with narrow forebody and wider postacetabular and postequatorial hind body. Acetabulum larger than oral sucker, wide apart from anterior extremity or subequatorial. Genital pore postacetabular, ventral to left caecum a little in front of anterior testis, postequatorial or pre-equatorial. Testes near one another separated by ovary in posterior half of body. Cirrus sac large, lying transversely or obliquely across entire breadth of extracaecal field. Vesicula seminalis biparate. Cirrus stout, spined. Ovary intertesticular. Uterus very short or moderately long. Metraterm well developed, armed. Vitellaria extending along great part of body from hinder end almost to anterior end. Two pairs of excretory longitudinal vessels extending along whole length of body joined anteriorly by transverse connecting vessels on each side. Parasitic in intestine of snakes and cocodiles.

Genotype: Harmotrema infecundum Nicoll, 1914.

## Helicotrematinae n. subf.

Subfamily diagnosis.—Harmotrematidae n. fam.: Body long, slender with uniform breadth. Acetabulum subequal to oral sucker, situated near anterior end, a little behind intestinal bifurcation. Oral sucker small. Pharynx proportionately to oral sucker large; oesophagus very short. Genital pore postacetabular, sinistral, submarginal at about half way between intestinal bifurcation and anterior testis. Testes entirely in anterior half of body, tandem, to left side i.e., to the side of genital pore. Cirrus sac curved to left side much in front of anterior testis, half way between it and intestinal bifurcation. Vesicula seminalis winding; prostatic complex and large armed cirrus contained within cirrus sac. Ovary intertesticular, quite apart from testes and in the same line with them. Vitellaria extending along caeca almost confluent posteriorly, leaving anterior part of body free. Uterus of moderate length, rectilineal, closely inside left caecum. Metraterm well developed, armed. A pair of longitudinal excretory vessels present on each side and united anteriorly behind pharynx. Parasitic in intestine of Lacertae.

Genotype: Helicotrema magniovatum Odhner, 1912.

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# ANTHRACNOSE DISEASE OF 'KARAUNDA' (CARISSA CARANDAS L.) CAUSED BY GLOEOSPORIUM CARISSAE AGARWAL

 $B_1$ 

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#### INTRODUCTION

In August, 1958 Carissa carardas (vern. 'Karaunda') plants were found suffering from leaf spot and die-back diseases at Jabalpur. Both young and old plants were affected. The causal organism was found to be Gloeosporium sp. which is known to cause anthracnose.

Lal and Singh (1953) reported Colletetrichum inamdarii Lal to cause anthracnose of Carissa carandas in Uttar Pradesh but since setae were not observed in naturally infected material or in culture at Jabalpur, the pathogen was Gloeosporium sp. Studies in the morphology and physiology of this fungus are reported in this paper.

# Symptoms of the disease:

The disease starts as small brown spots on either surface of the leaf. The spots increase in size and become circular to irregular. The central region of the lesions becomes pale grey with reddish brown margins. Spots may coalesce increasing the diseased surface. The plants begin to die backwards from the tip of a branch. The infected tip loses colour, becomes brown and extends backwards causing die-back. Later on the colour changes to whitish grey developing necrotic areas. The infected region is sharply demarcated from the healthy tissues.

# Morphology of the causal organism:

Acervulus broad, light brown, sub-epidermal,  $73\cdot 4-180\,\mu$  wide; conidiophores simple, nonseptate, hyaline; conidia hyaline, cylindrical to oblong, single celled,  $6\cdot 6-13\times 3\cdot 3-4\,\mu$ , average  $7\times 3\cdot 5\,\mu$ . (Fig. 1).



Fig. 1. Gloeosporium carissae Agaiwal. Acervulus, conidiophores and conidia.

<sup>\*</sup>Now known as Government Science College.

The spores in the present fungus are much smaller than those of Golletotrichum inamdarii on Carissa carandas described by Lal and Singh (1953). So far there is no record of any Gloeosporium on Carissa. Since specialization in Melanconiales is largely based on the host, the present fungus is being described here as a new species, namely Gloeosporium carissae Agarwal.

# Gloeosporium carissae Agarwal sp. nov.

Acervuli lati, pallide brunnei, subepidermali,  $73.4-180~\mu$  lati; conidiophori simplices, nonseptati, hyalini; conidia hyalina, cylindrica vel oblonga, unicellulata,  $6.6-13\times3.3-4~\mu$ , medietate  $7\times3.5~\mu$ .

The type specimen is deposited in the Herb. Crypt. Ind. Orient., New Delhi.

## MATERIAL AND METHODS

Isolations were made from spotted leaves and affected stems of *Garissa carandas* collected from different localities at Jabalpur. Isolations in every case yielded the same fungus. Single spore cultures were prepared that formed the parent culture employed in the present studies.

Pathogenicity tests were made in potted plants in the laboratory as well as on the old plants in the garden. Artificial inoculations were made in the young and old leaves, stems and fruits, ripe and unripe, in the injured and uninjured conditions. Injury to the leaves was caused by pricking them with a sterilized needle. Care was taken to avoid deep wounds and in no case it was allowed to reach the side of the leaf other than the one for which it was intended. Controls were maintained under identical conditions.

A small mass of mycellium mixed with spores formed the inoculum in the case of stems and fruits, For inoculating leaves following types of inoculums were tried:

- (1) Mass of mycelium and spores.
- (2) Spore suspension in sterilized water.
- (3) Sprinkling of powdered diseased leaves.

Preliminary observations have indicated that moisture appeared essential for a successful inoculation and therefore, moist cotton pads were kept on the inoculated regions. Reisolations were always made to confirm the results.

## Pathogenicity:

TABLE I

Showing infection of leaves of Carissa carandas by Gloeosporium carissae in two year old plants.

Type of inoculum	Leaf surface	Condition of leaf	No. in Old			nfected Young		infection Young
Mass of Mycellum	Upper	Injured	20	15	16	15	80	100
and spores		Uninjured	20	15	Nil	1	0	10
	Lower	Injured	20	20	17	18	85	90
		Uninjured	10	10	Nil	Nil	0 -	0

Type of inoculum	Leaf surface	Condition of leaf	No. i Old	noculated Young		infected Young	Percent i	infection Young
Spore	Upper	Injured	15	15	8	9	53.3	60
Suspension	· rr	Uninjured	20	20	Nil	Nil	0	0
Duspension	Lower	Injured	20	10	10	6 .	50	60
	Dower	Uninjured	20	20	2	3	10	15
Sprinkling	Upper	Injured	20	20	5	7	25	35
powder of	@ppcr	Uninjured	10	10	Nil	Nil	0	0
infected	Lower	Injured	20	20	6	6	30	30
leaves	TOWCI	Uninjured	15	15	Nil	Nil	0	0

In all cases infection could be noticed within four days of inoculation. Comparing the three types of inoculums it is evident that mass inoculum is the most successful followed by spore suspension and powder of infected leaves.

It appears that only injured leaves can be infected. However, infection was observed sometimes in the uninjured cases. Probably such leaves might have been injured naturally. Both the surfaces responded.

Percentage infection in the young leaves was slightly more than in the old ones. It was observed that the spread of the disease was comparatively faster in the young ones than the old.

The uninjured stems gave negative results whereas the injured ones were

Infection experiments performed in the garden on old trees gave similar results. Old stems however, failed to catch the infection.

Pathogenicity was also tested both on ripe and unripe fruits. The uninjured ones gave negative results. The infection and the spread of the disease in the unripe fruits was much less compared to that of the ripe ones. The infection appeared on the third day in the ripe ones and on the fourth day in the unripe ones.

Gloeosporium carissae was invariably obtained on reisolations in all the cases.

# Host Range:

The fungus did not infect Musa paradisiaca, Citrus sp. Carica papaya but infected Psidium guajava, Anthocephalus cadamba, Artocarpus integrifolia and Capsicum annum.

## Control:

It was found that 0.1% copper sulphate sprays, as suggested by Lal and Singh (1953) could be used effectively in cases of severe infection.

# Growth and Nutrition:

The growth of the fungus was studied on the following solid and liquid media.

- 1. Asthana and Hawker's medium—Potassium nitrate 3.5 gms., potassium dihydrogen phosphate 1.75 gm., magnesium sulphate 0.75 gm., glucose 5 gm., water 1000 ml.
- 2. Brown's starch medium—Glucose 2 gm., asparagin 2 gm., potassium dihydrogen phosphate 1.25 gm., magnesium sulphate 0.75 gm., soluble starch 10 gm., water 1000 ml.

- 3. Czapek's medium—Sodium nitrate 2 gm., potassium dihydrogen phosphate 1 gm., potassium chloride 0.5 gm., magnesium sulphate 0.5 gm., ferrous sulphate 0.01 gm., sucrose 30 gm., water 1000 ml.
- 4. Coon's medium—Maltose 3.5 gm., asparagin 0.25 gm., potassium dihydrogen phosphate 1.25 gm., magnesium sulphate 0.5 gm., water 1000 ml.
- 5. Richard's medium—Potassium nitrate 10 gm., potassium dihydrogen phosphate 5 gm., magnesium sulphate 2.5 gm., sucrose 50 gm., water 1000 ml.
- 6. Potato-glucose medium—200 gms. of potatoes were cut in to small pieces and boiled for half an hour in some distilled water. 20 gms. of glucose was added to the filtrate and the total volume of the medium was adjusted to one litre.
- 7. Host leaf decoction—90 gms. of Karaunda leaves were boiled in distilled water for one hour, filtered through cloth and the total volume made to 600 ml.

Pyrex glassware, purest available chemicals and distilled water were used in all the experiments. In each conical flask of 150 ml. capacity 50 ml. of various liquid media were taken and sterilized at 15 lbs. pressure for 15 minutes. Seeding of the culture flasks was done by inocula of equal size, cut by the agar disc method, from seven-day-old cultures grown in petri dishes. The flasks were incubated at room temperature for 15 days.

At the end of the incubation period the cultures were filtered on previously weighed Whatman filter papers No. 12. The filter papers were dried at 70°C for three days. Only average values of the four replicates to the nearest mg. have been recorded.

TABLE II

Dry weight, sporulation and size of spores on different media.

Medium	Dry weight (mg.)	Sporulation	Size of spores in $\mu$
Asthana Hawker	200 .	Good	9·8–19·7 × 1·6–3·3
Brown's starch	334	Good	9·8–16 × 1·6–4·9
Czapek's	3.12	V. poor	0 0-10 × 1 0-4.9
Coon's	246	Good	$6.6-16.4 \times 2.5-3.3$
Richard's	298	Moderate	$6.6-16.4 \times 1.6-4.9$
Potato-glucose	310	Moderate	$6.6-16.4 \times 3.3$
Host leaf extract	138	Good	$6.6-13 \times 1.6-4.9$

The best dry weight of the fungus was obtained on Brown's starch medium followed by Czapek's, potato-glucose, Richard's, Coon's, Asthana Hawker and host leaf extract.

Sporulation was good on Asthana Hawker, Brown's starch, Coon's and host leaf extract. It was moderate on Richard's and potato-glucose and very poor on Czapek's. The size of spores were not appreciably affected by the different media.

Growth and sporulation of the fungus was also studied on solid media in petri dishes. Solidification was done by mixing 2% agar. To measure the linear advance of the fungus the average of the minimum and the maximum diameter of its daily growth was recorded.

TABLE III

Diameter of colony in centimeters and sporulation on different solid media.

Medium				Days					Sporulation
viedium	2nd	3ra	4th	əth	6th	7th	8th	9th	o por diation
Asthana Hawker	1.2	2.1	2.8	3.4	4.0	4.6	5.5	7:1	Good
Brown's starch	1.1	1.9	2.6	3.2	3.6	4.1	5.0	6.2	Good
Czapek's	1.1	2.2	3.0	3.8	4.6	5.4	6.4	7.5	Poor
Cloon's	1.2	2.0	2.8	3.6	4.4	5.0	5.5	6.8	Good
Richard's	1.8	2.7	3.6	4.4	5.2	6.0	6.8	<b>7·</b> 8	Moderate
Potato-glucose	1.4	2.6	3.5	4.3	5-1	6.1	7.1		Moderate
Host leaf extract	0.6	2.0	2.9	3.5	4.4	5-1	5.7		Good
Host leaf extract	0.6	2.0	2.9	3.5	4.4	5-1	5.	7	7

On the basis of radial advance the different media may by arranged in the following order:

Potato-glucose, Richard's, Czapek's, Host Ieaf extract, Asthana Hawker's, Coon's, Brown's starch.

# pH in relation to growth

To study the effect of pH the basal medium (Brown's starch) was adjusted with HCl or NaOH before autoclaving. The pH was determined with a Beckman electronic pH meter.

TABLE IV

Dry weight, final reaction of the medium after the incubation period and sporulation at different pH.

Initial pH	Dry weight (mg)	. Final pH	Sporulation
2.8	198	5.6	Moderate
3.6	283	6.5	Good
4.4	312	6.8	$\operatorname{Good} olimits$
4.8	340	6.8	Good
5.6	329	6.8	$\operatorname{Good}$
7.0	290	7.5	Good
8.0	168	8.0	Good
10.5	Nil		

The fungus could grow on a wide pH range (2.8-8.0), the optimum being 4.8. The sporulation was good at pH 3.6-8.0.

# Temperature relation:

TABLE V

Dry weight and sporulation at different temperatures.					
Temperature in C.	Dry weight (mg)	Sporulation			
6	Nil	• •			
25	288	$\operatorname{Good}$			
30	336	Good			
35	210	Good			
40	Traces				

The fungus did not grow at 6 and 40°C. It developed good growth as well as good sporulation between 25—35°C, the optimum being 30°C.

## Carbon requirements:

The carbon requirements were studied by substituting various carbon compounds for maltose in the Coon's medium. Brown's starch medium was not selected here because it contained starch and glucose as carbon sources. The concentrations of different compounds were such that they resulted in the same amount of carbon as that present in 3.5 gm maltose, except for starch, which was added in the same quantity as maltose. The pH of different media was adjusted to 4.8.

TABLE VI

Dry weight and sporulation on different carbon compounds.

Carbon compound	Dry weight (mg)	Sporulation		
Rhamnose	226	Good		
Xylose	240	$\operatorname{Good} olimits$		
Glucose	264	Good		
Galactose	198	Poor		
Maltose	250	$\operatorname{Good}$		
Sucrose	257	Moderate		
Lactose	242	Moderate		
Starch	300	$\mathbf{Good}$		
Mannitol	190	Poor		
Sorbitol	228	Poor		
Dulcitol	202	Poor		
Oxalic acid	Traces	• •		
No carbon source	Nil.	• •		

There was no growth in the absence of carbon from the medium. The best growth was on starch followed by glucose, sucrose, maltose, lactose, xylose, sorbitol rhamnose, dulcitol, galactose and mannitol. Growth was only in traces in oxalic acid.

Sporulation was good on rhamnose, xylose, glucose, maltose and starch, moderate on sucrose and lactose and poor on galactose, mannitol, sorbitol and dulcitol.

# Nitrogen requirements:

The different nitrogen compounds were added singly to Brown's starch medium minus asparagin in amounts which supplied the same weight of nitrogen as that present in 2 gm/l asparagin. The amount of peptone added was equal to that of asparagin. pH of all the media was adjusted to 4.8.

TABLE VII

Dry weight and sporulation on different nitrogen compounds.

Nitrogen compounds	Dry weight (mg)	Sporulation
Sodium nitrate	192	Good
Potassium nitrate	186	Good
Ammonium nitrate	230	Poor
Ammonium chloride	276	V. Poor
Ammonium sulphate	268	V. Poor
Ammonium phosphate	259	Good
Sodium nitrite	Nil	• •
Potassium nitrite	Nil	
Urea	173	Moderate
Acetamide	200	Poor
Peptone	327	Abundant
A sparagin	340	Good
No nitrogen	Nil	• • •

The best dry weight of the fungus was obtained on asparagin followed by peptone, ammonium chloride, ammonium sulphate, ammonium phosphate, ammonium nitrate, acetamide, sodium nitrate, potassium nitrate and urea. There was no growth on sodium nitrite, potassium nitrite and on the medium devoid of any nitrogen compound.

Sporulation was abundant on peptone. It was good on sodium nitrate, potassium nitrate, ammonium phosphate and asparagin. Urea induced moderate sporulation which was poor on ammonium nitrate acetamide. It was very poor on ammonium chloride and ammonium sulphate.

## Discussion and Conclusions:

Pathogenicity experiments on leaves, stems and fruits indicated that Gloeosporium carissae is a wound parasite. The fungus can attack plants other than Carissa carandas also. On comparing the diametrical advance of the fungus with the dry weight determinations, it is evident that the order of preference of different media is not the same in the two cases. One of the essential defects of a solid medium is that it neglects the thickness of the colony or the amount of mycelium produced. According to Lilly and Barnett (1951) the rate of linear growth of some fungi has little relation to the composition of the medium. The rapid extension of mycelium on water agar may serve as a familiar example. The dry weight results are therefore more reliable.

Brown's starch medium supported the best dry weight as well as good sporulation. It may be because of asparagin, which was found to be best nitrogen source, and comparatively a greater amount of carbon in the medium.

Fungi can grow within a wide range of pH and they prefer slightly acidic media (Hawker, 1950). Gloeosporium carissae grows at pH 2·8-8·0, optimum 4·8, and appeared to prefer acidic to alkaline media. In this respect it differs from Golletotric um inamdarii for which alkaline reactions seem to be more suitable than acidic reactions (Lal and Singh, 1953).

The fungi as a result of their metabolic activity change the pH of the medium on which they grow (Hawker, 1950; Agarwal. 1958 a-c; Agarwal and Shinkhede, 1959). Ramkrishnan (1941) working with Colletotrichum observed that whatever the initial pH the final values were always in the region of neutrality. Gloeosporium carissae increased the final reaction of the culture medium, but there was no change at pH 8·0. Lilly and Barnett (1947) ascribe these changes in pH to the metabolic activity of the fungus upon the carbohydrates of the medium.

The fungus has a wide range for its carbon requirements and the carbon from different sources is utilized fairly well. Brock (1951) remarked that while interpreting the data on growth in relation to the supply of different carbon compounds, it is pertinent to bear in mind that growth is a very complex physiological process and as such its magnitude is never determined by a single nutritional factor, but on the other hand, a set of interdependent environmental factors are always involved. In the present studies only the source of carbon was varied; asparagin was the sole source of nitrogen and other factors were kept uniform.

The growth of the fungus on starch was the best. The utilization of higher complex carbohydrates by fungi may largely depend upon their synthesis of suitable hydrolytic enzymes. According to Lilly and Barnett (1951) only those fungi which produce amylase are able to utilize starch; this ability is common but not universal among fungi. Starch has been found to be a good carbon source for many fungi (Mehrotra, 1951; Bilgrami, 1956; Tandon and Agarwal, 1957; Thind and Randhawa, 1957; Tandon and Bhargava, 1960).

Gloeosporium carissae can utilize nitrogen from a number of compounds. The best growth was obtained on asparagin.

Fungi rarely give appreciable amount of growth on nitrites which are generally considered to be toxic to fungi (Tandon and Bilgrami, 1954; Tandon and Grewal, 1956; Agarwal, 1958 d). The present organism could not utilize nitrite nitrogen. There are, however, records of some fungi which can grow on nitrites (Thind and Sandhu, 1956; Tandon and Agarwal, 1953). Brock (1951) has suggested that the toxicity of nitrites may be associated with pH range specially because most of the fungi are cultured in the acid range where according to Gochrane and Conn (1950) and Nord and Mull (1945) nitrites are usually in the form of undissociated nitrous acid which has an unfavourable effect on growth.

A leaf spot and die-back disease of Carissa carandas L. at Jabalpur caused by a new species of Gloeosporium, named as Gloeosporium carissas, has been described.

Infection experiments were carried out and the pathogenicity on leaves, stem and fruits was established. The fungus was found to be a wound parasite. Cross inoculations on Musa paradisiaca, Citrus sp. and Carica papaya gave negative results. The organism could infect Psidium guajava, Anthocephalus cadamba, Artocarpus integrifolia and Capsicum annum.

Physiology and nutrition of the fungus have been studied. It grows on pH 2.8-8.0, optimum being 4.8. The optimum temperature was 30°C.

Starch was the best source of carbon followed by glucose, sucrose, maltose, lactose, xylose, sorbitol, rhamnose, dulcitol, galactose and mannitol.

Asparagin was the best source of nitrogen followed by peptone, ammonium chloride, ammonium sulphate, ammonium phosphate, ammouium nitrate. acetamide, sodium nitrate, potassium nitrate and urea. There was no growth on nitrites of sodium and potassium.

#### ACKNOWLEDGMENT

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# SOME NEW RECORDS OF ASCOMYCETES FROM INDIA

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The author proposes to describe in this paper seven interesting ascigerous moulds which were obtained from different substrata in Varanasi. The first three were collected on decaying twigs in 1958 during routine collection of saprophytic fungi in the rainy season and the others were isolated at various depths from different grassland soils. Soil samples were collected with aseptic precautions and were examined by cultural method for the presence in them of fungi. Methods adopted for soil sampling and medium used for isolation were same as described by Dwivedi\* (1958). The following is an account of these fungi:

1. Tryblidiella rufula (Spreng.) Sacc. var. microspora Ell. and Ev. Deight, F. C. 1936. Rep. Dep. Agric. S. Leone, pp. 18-22.

Apothecia superficial, sometimes innate and concrete with the epidermis, oblong or linear, typically black, carbonous, opening by a narrow cleft, separate, very rarely caespitose, 0.5-10 mm in length; asci elongated, cylindrical, hyaline to yellowish when young, brown to deep brown at maturity, eight spored, 126-162 x 9-10.8 μ; paraphyses hyaline; ascospores oblong, obliquely monostichous, three septate, yellowish when young and deep brown at maturity,  $18-21.5 \times 7.2-9 \mu$ . (Text-fig. 1).



1. Tryblidiella rufula (Spreng.) Sacc. var. microspora Ell. and Ev. A. Apothecia; B. Ascus; C. Ascospores.

It was collected on dead twigs of Citrus medica Linn. (Herb. I. M. I., Kew, No. 74888).

<sup>\*</sup>Dwivedi, R. S. 1958. Some soil fungi of Varanasi. Proc. Nat. Acad. Sci. India, 28(B): 331-339.

## 2. Otthia crataegi Nits.

Saccardo, P. A. 1882.. Syll. Fung. I. pp. 735.

Perithecia erumpent, caespitose, ramicole, minute, globose, ostiolate, 0.5 mm in diameter; asci stipitate, cylindrical, eight spored,  $120.5-180.6\times15-27~\mu$ ; paraphyses present; ascospores uniseriate, ovate to oblong, two celled, constricted at the septum, apices blunt,  $24-33.5\times9-12~\mu$ . (Text-fig. 2).

It was collected on a decaying twig and is deposited in Botany Department, B. H. U.

# 3. Didymella andropogonis Ell. and Ev.

Perithecia membranous, erumpent, globose, caespitose, sometimes separate, black; asci hyaline, elongate, eight spored,  $63-90\times3\cdot5-5\cdot5$   $\mu$ ; paraphyses present; ascospores uniseriate, hyaline with greenish tinge, two septate, apices blunt,  $10\cdot8-12\cdot5\times3\cdot6$   $\mu$ . (Text-fig. 3).



Fig. 2. Otthia crataegi Nits.

A. Ascus; B. Ascospores.

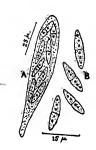


Fig. 3. Didymella andropogonis Ell. and Ev. A. Ascus; B. Ascospores.

It was collected on dead leaves of Vetiveria zizanioides Nash. (Herb. I. M. I., Kew, No. 74889).

# 4. Penicillium spiculisporum Lehman

Lehman, S. G. 1920. Mycologia, 12: pp. 268-274.

Colonies on Czapek's agar growing very slowly attaining a diameter of 1.5 cm after two weeks at 25°C., consisting of a tough mycelial felt; colonies pure white at first becoming slight yellowish at maturity, azonate; reverse light-yellowish; conidial structures usually not produced, if present a few in number specially at the margin, not affecting the colony appearance; conidiophores smooth walled, hyaline, short  $14\cdot4-54\times1\cdot8-2\cdot7$   $\mu$ , bearing a single verticel of three to five phialides, occasionally only one or two phialides on the conidiophores, frequently conidiophores producing a side branch bearing a single chain of conidia; phialides  $10\cdot8-18\times2-2\cdot7$   $\mu$ , tapering at the end; conidia globose to sub-globose  $2\cdot4-4\times2-3\cdot2$   $\mu$ ; perithecia developing after ten to twelve days, white to light-yellowish, spherical to globose, sometimes oblong, variable in size, upto 360  $\mu$  in diameter;

wall composed of interwoven hyphae; asci globose to oval, hyaline, eight spored, 6.4-8  $\mu$  in diameter; ascospores elliptical, hyaline, thick and smooth walled in the beginning becoming finely spinulose at maturity,  $3\cdot2-4\times2-2\cdot4$   $\mu$ . The spines visible only under oil immerson. (Text-fig. 4).

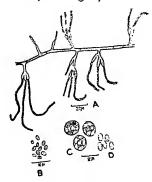


Fig. 4. Penicillium spiculisporum Lehman

A. Ahypha bearing conidiophores and chains of conidia; B. Conidia; C. Asci; D. Ascospores.

The fungus was created by Lehman (1920) isolated from rootlets of apparently healthy cotton plants from a field in Anson County, N. C. It agrees with the description given by him. (Herb. I. M. I., Kew, No. 76551)

## 5. Penicillium brefeldianum Dodge

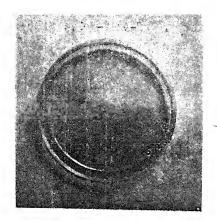
Syn. Carpenteles brefeldianum (Dodge) Shear

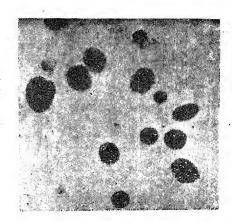
Dodge, B. O. 1933. Mycologia, 25: pp. 90-104;

Emmons, C. W. 1935. Mycologia. 27: pp. 128-150.

Colonies on Czapek's agar growing slowly, attaining a diameter of one cm. in a week; mycelium whitish becoming cream coloured at maturity; reverse brownish; perithecia developing after a week, sclerotia-like in the beginning, asci formation after two weeks, light-orange, globose to sub-globose, superficial, 90–180  $\mu$  in diameter when globose, and upto  $180\times162~\mu$  when sub-globose, asci numerous in each perithecium, hyaline, globose, eight spored, 64–8  $\mu$  in diameter; ascospores globose to slightly elliptical, hyaline, finely echinulate, 3·1–4  $\mu$  in diameter, with faint equatorial furrow.

The perithecia at first consist of a solid mass of pseudoparenchymatous tissue with little or no differentiation except for the somewhat thicker walls of the cells of the outer layer. The ascogenous system occupies a central position and gradually increases by disorganization of the surrounding tissue. When perithecia are matured, numerous globose asci are formed in them. These observations are in accordance with the observations made by Dodge (1933). No mention was made in his description regarding the presence of equatorial furrow on the ascospore. Later on Emmons (1935) figured the ascospore with a faint suggestion of an equatorial furrow. The author also observed such furrow on the ascospores but this character is very rare. During the course of the study conidial structures were not observed clearly. The perithecial development dominates the appearance of the colonies. (Text-fig. 5).





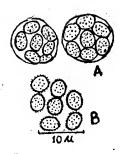


Fig. 5. Penicillium brefeldianum Dodge.

I. Photograph of ten days' growth of a colony showing perithecia.

II. Photomicrograph of perithecia.
III. A. Asci; B. Ascospores.

Its sub-culture was sent to the Commonwealth Mycological Institute for confirmation of the identification. Mr. Elphick's remark, regarding this fungus is 'The spherical sclerotial bodies produce asci after several weeks in culture. Repeated attempts to induce the formation of conidia have failed, but nevertheless the perithecia and ascospores are sufficiently characteristic to enable this fungus to be named with fair certainty." (Herb. I. M. I., Kew, No. 76550).

# 6. Penicillium javanicum Van Beyma

Syn. Carpenteles javanicum (Van Beyma) Shear

Emmons, C. W. 1935. Mycologia, 27: pp. 145-146.

Colonies on Czapek's agar growing fairly rapidly attaining a diameter of 2.5-4 cm. in 8-10 days at 25°C, comparatively thin, consisting of tough, closely textured mycelial felt, conspicuously furrowed in a predominantly radial patten with raised central area; colour of the colony at first white, then olive-buff, at maturity fawn (Colour Pl. 14A, 7-'Dictionary of Color' Maertz and Paul, 1930) in

centre of the colony. Conidial structure minute, limited in number, not influence ing the colony appearance, perithecia abundantly produced, dominating the growth of the colony and imparting granular appearance to the latter, yellowish brown in colour; exudate abundantly produced in rich brown shades, becoming deep purple brown in old stage; odour lacking; reverse of the colony, at first yellowish-brown, then maroon (Colour Pl. 7L, 7) with age; penicillia strictly monoverticillate, very few in number, restricted to the margin of the colonies; conidiophores arising mostly as branches from aerial hyphae, up to 36  $\mu$  in length adn 2  $\mu$  in diameter, unbranched, smooth walled; sterigmata borne in groups of 2-6, measuring from  $7\cdot2-10\cdot8\times2-2\cdot7~\mu$ ; conidia globose to sub-globose, greenish when in mass, mostly upto  $2\cdot4~\mu$ , occasionally upto  $3\cdot2~\mu$  in diameter. Perithecia abundantly produced, at first sclerotia-like, globose to sub-globose, sometimes angular, 120-320 \( \mu\_{\text{o}} \), in the beginning consisting of heavy walled parenchyma-like cells within, ripening very late, developing asci and ascospores after three weeks; asci borne as lateral buds from the fertile hyphae within the perithecium, globose to sub-globose at maturity, 5.6-8 \( \mu \) in diameter; eight spored; ascospores heavy walled, showing surface irregularities and an equatorial furrow, globose to sub-globose, mostly lenticular,  $3\cdot 2-2\cdot 4\times 2\cdot 4-1\cdot 6$   $\mu$ . (Text-fig. 6).

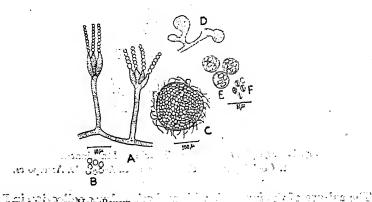


Fig. 6. Penicillium javanicum Van Beyma

- A. Conidiophores bearing chains of conidia.
- B. Conidia; C. A perithecium; D. Young asci; E. Mature asci; F. Ascospores.

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Numerous strains of this species have been recognised by Raper and Thom (1949). Van Beyma (1929) neither reported any irregularities on the spore surface nor gave any indication of equatorial line and asci with eight spores. But later on Raper and Thom (1949) reported the presence of these characters in the fungus. The author's observations are in conformity with the findings of Raper and Thom. Further, the fungus in question differs from previously described strains of this species in producing very large perithecia and in occasional occurrence of large conidia. Undoubtedly it is a new strain not reported so far.

The culture of the fungus is deposited in Botany Department, Banaras Hindu. University.

7. Aspergillus montevidensis Talici and MacKinnon Thom, C. and Raper, K. B. 1945. A Manual of Aspergilli, p. 125. Colonies on Czapek's agar spreading very slowly, at first green due to conidial heads, later on becoming yellow due to development of perithecia; reverse yellowish green; conidial heads small, columnar with a few conidial chains; conidiophores yellowish, smooth walled,  $220-270~\mu$  long and  $5\cdot4-7\cdot2~\mu$  broad, frequently very short when borne on aerial mycelium, broadening below the conidial head; vesicles hemispherical to dome shaped, commonly brown with tinge of green colour,  $10\cdot8-18~\mu$  in diameter, occasionaly large; sterigmata in one series, relatively short and thick,  $7\cdot2-9\times3\cdot3\cdot6~\mu$ ; conidia globose, to sub-globose variable in size, roughened,  $5\cdot4-7\cdot2\times3\cdot6-5\cdot4~\mu$ , yellowish-green in mass. Perithecia abundant, yellow in young stage becoming yellowish-brown at maturity, varying in size and irregular in shape, at first containing parenchymatous cells, later developing asciand ascospores; asci globose, yellowish,  $9-12\cdot6~\mu$  in diameter; ascospores lenticular, yellowish, roughened, furrowed, with acute and irregular ridges, mostly  $4\cdot8-5\cdot6\times3\cdot2-4~\mu$ . (Text-fig. 7).

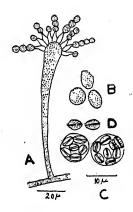


Fig. 7. Aspergillus montevidensis Talici and Mackinnon.
A. Conidiophore; B. Conidia; C. Asci; D. Ascospores.

The culture of the fungus is with author's culture collection in Botany Department, Banaras Hindu University.

## SUMMARY

This paper deals with seven ascigerous fungi, viz. Tryblidiella rufula (Spreng.) Sacc. var. microspora Ell. and Ev., Otthia crataegi Nits, Didymella andropogonis Ell. and Ev., Penicillium spiculisporum Lehman, P. brefeldianum Dodge, P. javanicum Van Beyma, and Aspergillus montevidensis Talici and MacKinnon, which are new records from India. The first three were collected on decaying twigs and the rest were isolated at various depths from grassland soils.

In conclusion the author expresses his gratefulness to Dr. R. Y. Roy for guidance, Dr. J. C. F. Hopkins, Director, Dr. Booth, Mr. Elphick of C. M. I., Kew, for kind suggestions and help in identifying and confirming the identification of some of the species, and Prof. R. Misra, F. N. I. for all the facilities.

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## L. N. JOHRI

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[Received on 11th January, 1961]

The material, included in this paper is collected from the birds shot in Delhi. The author expresses his sincere thanks to the Council of Scientific and Industrial Research for the financial assistance in course of the present investigations. All measurements unless mentioned otherwise are given in mm.

Family ANOPLOCEPHALIDAE Subfamily ANOPLOCEPHALINAE

Fuhrmann 1907. Blanchard 1891.

Killigrewia

Meggitt 1927.

Meggitt (1927) created this genus with K. pamelae as the type species and also added K. frivola. Fuhrmann (1932) regarded Killigrewia as a synonym of Aporina Fuhrmann 1932. Johri (1934) while reporting Killigrewia from Lucknow (India) discussed the validity of these genera and definitely recognized Killigrewia as a definite genus. Subsequently Yamaguti (1935) added K. oenopopeliae and K. streptopeliae from Japan. Sharma (1944) reported Nepalesia jeodhii from Nepal. This genus Nepalesia is rightly considered by Yamaguti (1959) as a synonym of Killigrewia. thereby including K. jeodhii (Sharma, 1944) in the list. K. indica n.sp., is further added in this communication.

## Killigrewia indica n.sp.

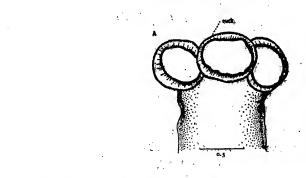
Host: Cloropis auriforns (Temm. & Laug).

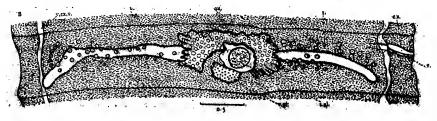
Maximum length is 100.5 and the greatest breadth is 4.65 (mature segments) and 6.0 (gravid segments). All the segments are very much broader than long. Genital pores are irregularly alternating and are located at one-third anterior margin of the proglottis.

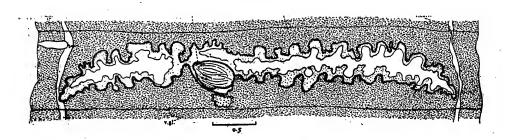
Scolex is 0.73 long and 0.64 broad and bears four unarmed suckers measuring 0.6-0.7 in maximum diameter. The suckers are greatly protruding out from the general part of the scolex and at this level the scolex attains a breadth of 1.55.

There are 91-100 testes which are arranged in two groups lateral to female reproductive organs, 37-41 on the poral side and 54-59 on the aporal side. Some of these may come in close contact with lateral lobules of the ovary. Cirrus sac measures  $0.32 \times 0.04$  (mature segment) and  $0.58 \times 0.08$  (gravid segments) and is just reaching ventral longitudinal excretory vessel. Cirrus is simple without any armature. Ovary is distinctly bilobed, each lobe with large number of lobulations mainly on its lateral borders. It is slightly poral and occupies nearly two thirds of the length of the proglottis, and measures 1.2 in maximum breadth. A compact vitelline gland, situated posterior to ovary, is provided with small lobulations, and measures 0.43 in diameter. A shell gland lies in between the ovary and the vitelline gland and measures 0.25 in diameter. Receptaculum seminis is very prominent and measures 0.4 (mature segments) and 0.55 (gravid segments). A simple vagina is opening in a shallow cloaca posterior to cirrus sac. Uterus is at first a simple transverse tube which is very prominent in the mature segments,

commencing in close contact with the lateral sides of the ovary and extending upto the longitudinal excretory vessels on either side. At a later development it produces backward and forward numerous pouches separated from each other by dorsoventral septa and it is always limited within the longitudinal excretory vessels. Eggs measure 0.015–0.018 in diameter.







# Killigrewia indica n.sp.

A. Scolex. B. Mature segment. C, Gravid segment.

## ABREVIATIONS USED

c.s.—cirrus sac; ov.—ovary; r.s.—receptaculum. seminis; s.gl.—shell gland; suck.—sucker; t.—testis; u.—uterus; v.—vagina; v.ex.v.—ventral longitudinal excretory vessel; v.gl.—vitelline gland.

Killigrewia Meggitt, 1927

	Egg size in	~	53	51–57 39–42	1	30–33 r × 22–24	15-18 dia.
	Cirrus	~	armed	with cuticular hairs	armed	with cuticular hairs	no spines (simple)
	Vesicula seminalis	present	absent	present	~	present	absent
	R. S. size in	large	absent	200–300 diameter	large and spherical	120-250	400-500
	Ovary	slightly poral, alternating from side to side with genital pore	central	anterior half of proglottid, approximately central	rather poral	poral	poral, occupying 4/4th length of the segment.
	Genital pore (position)	anterior quarter of proglettid margin	1/3rd anterior	anterior half of proglottid margin	anterior third of proglottid margin	middle of anterior half of proglottid margin	anterior one- third of proglottid margin
	Cirrus sac (extent) in relation to long, ex. v.	extending to or not quite to	reaching	reaching	extending to or just past	reaching or not quite	Ç
	Girrus sac size in $\mu$	220-250 X 66110	320×40 (mat. segt.) 580×80 (gr. segt.)	230—250 X 50—75	180×00	200—250 X 80—100	320—580 X 40—80
	Testes (No.)	103–145 (113 to 154) per Johri	91-190 3	20	65	70-120	82-86
*	Species	K. frivola Meggitt, 1927	K. jeodhii (Sharma, 1944)	K. oenopopeliae Yamaguti, 1935	K. pamelae Meggitt, 1927	K. streptopeliae Yamaguti, 1935	K. indica n.sp.

Present form differs from K. oenopoleliae Yamaguti 1935 and K. streptopeliae, Yamaguti 1935 in the number and the range of the testes, size of the cirrus sac and the receptaculum seminis, absence of spines and cuticular hairs on the cirrus and the smaller size of eggs. It is easily distinguished from K. frivola Meggitt 1927 on account of small number of testes, relative and absolute size of cirrus sac, the presence of receptaculum seminis and absence of a vesicula seminalis. Again the present form is clearly separated out from K. pamelae Meggitt 1927 by the larger number of testes and their position in relation to ovary (in K. pamelae testes are arranged in two lateral fields and the aporal group is further away from ovary), larger size of the cirrus sac and in absence of the spines on the cirrus. The relative and absolute size of cirrus sac, simple cirrus without any armature, larger number of testes, slightly poral location of the ovary, presence of a distinct receptaculum seminis, the smaller eggs and the absence of the spines on the cuticle in the present form clearly separate out K. jeadhii (Sharma, 1944).

It is, therefore, essential to create a new species K indica to accommodate the present form.

#### SUMMARY

A review of the genus Killigrewia Meggitt, 1927 together with its present status and validity are explained.

#### Killigrewia indica n.sp.

Host: Cloropis auriforns (Temm. & Laug).

Maximum length 100.5. Greatest breadth 4.65 (mature segments) and 6.0 (gravid segments). All segments much broader than long. Genital pores irregularly alternate and located at one-third of the margin. Scolex 0.64 in maximum diameter. Suckers 0.6-0.7 in diameter. Testes 91-100 arranged in two lateral groups: poral and aporal groups constitute 37-41 and 54-59 respectively. Cirrus sac measuring 0.32 × 0.04 (mature segments) and 0.58 × 0.08 (gravid segments) and reaches ventral longitudinal excretory vessel. Cirrus simple without any armature. Ovary slightly poral and is provided with numerous lobulations from its lateral borders. Vitelline gland with small lobulations measuring 0.43 in diameter. Shell gland measures 0.25 in diameter. Uterus with numerous backward and forward 25-30 diverticulae from the main uterine stem. Eggs measure 0.015-0.018 in diameter.

The present form represents outstanding characters on account of absolute and relative size of cirrus sac, large number of testes, presence of a distinct receptaculum seminis and smaller eggs. It has been compared with the allied forms and is separated out as a distinct and new species.

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### BIOLOGY OF BIMBA TOOMBILL GROVER (CECIDOMYIIDAE: NEMATOCERA: DIPTERA)<sup>2</sup>

By

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[Received on 3rd March, 1962]

#### INTRODUCTION

Ever since Schiner (1868) inaugurated and Kieffer (1905) elaborated the study of gall-midges in this country, the work on the systematics of the Cecidomyidae continued to progress steadily although at a very; slow speed. But, however, little attention has been paid to the study of the Biology of these economically important insects. It is evident from the available records that only a few attempts have been made to study the Biology of midges. Rao. Y. R. (1917; Ayyar, T. V. (1921), Ghosh, C. C. (1921), Pruthi, H. S. (1937), Sen, P. (1938-1939) and Sen, A. C. (1928), 1939. (1952-1953) are some of the workers who have paid attention towards this aspect of study but even these studies are not complete. The present study, therefore, was undertaken to present as complete an account of Biology of the gall-midges as possible. Biology of Bimba toombii Grover is the first step of the series.

Bimba gen. nov. belongs to the tribe Lasiopterini of subfamily Itonididinae family Cecidomyidae (Itonididae). It comes very close to Protoplonyx Felt with which it is readily distinguished by the dentate claws on all legs, the wing ventation and the structure of the genitalia. It also bears superficial resemblance with Neoprotoplonyx Rao (1950) by the presence of quadriarticulate palpi and the dentate claws, but is easily separated from it by the wing venation, the structure of the antennal segments and the ovipositor. The genus has been named after 'Bimba', the Sanskrit name of Coccinia indica with toombii as its new species.

Specific diagnoses are given below:

Male: (Plate I, B). Body 0.8 mm. long. Eyes confluent above, palpi quadriar ticulate, antenna 2+12, one fourth the length of the body, third and fourthantennal segments not fused together, segments sessile with low circumfila. Wings hyaline, length twice the breadth.  $R_5$  very close to costa, vein  $M_{1+2}$  present and curved, vein Cu simple. Claw unidentate on all legs, empodium as long as claw. Abdomen yellowish-orange with black hairs. Genitalia; basal clasp segment without basal lobe, terminal clasp segment slender, shorter than basal clasp segement. Dorsal plate deeply and narrowly incised, lobes rounded, ventral plate shorter than dorsal plate and linear, rounded apically. Harpes bilobed and sclerotized, margins beset with recurved setae.

Female: (Plate I, A). Body 1.5 mm. long, darker than male. Antenna 2+16; 3rd and 4th antennal segments not fused together. Ovipositor of usual Lasiopteran type, one-fourth the length of the body, other general characters similar to those of the male.

Studies of gall midge (Itonididae-Cecidomylidae: Diptera) unpublished. 2. The work was completed under the tenure of Government of India Junior Research Fellowship. 

#### METHOD AND MATERIAL

Several hundreds of matured galls were collected from the various localities of Allahabad to study the Biology and Taxonomy. The midges were reared in rearing cages in the laboratory. The midges and the parasites that subsequently emerged were collected daily and the usual records including sex ratio at the time of emergence were taken. Similarly, hundreds of galls were dissected to examine the tunnels and to collect different larval stages. The adult flies and larvae thus collected from the cages were preserved and kept for subsequent use. A number of live midges were kept for breeding experiments.

The plucked galls were placed in the rearing chambers in the laboratory. Each consisted of a lamp glass the upper end of which was covered tightly with a muslin cloth or with fine nylon net, leaving a slit in the middle. This slit was closed with cotton wool. The lamp glass was kept on ordinary white plate on which the base of the chimney fitted tightly. Sand or fibres were not used in the present case because the larvae pupate inside the galls. Such rearing chambers were kept in rearing cages placed in open varandas of the laboratory. On emergence the flies were removed one by one through the slit with an alcoholwetted brush, and were collected in a tube containing a mixture of 80% alcohol and a small quantity of pure glycerine at the rate of a drop or two per small tube.

To study the copulation, oviposition and hatching of the eggs in normal conditions, an experimental field cage was set up. This cage is  $6 \times 6 \times 3$  feet with walls and roof made up of fine wire guaze (Plate II, F). The cage was placed out door in the field to maintain out-door humidity and temperature. Flower pots with host plant were placed inside the cage and allowed to grow without any chance of infection. When sufficient midges were collected, they were released in the field-cage. Copulation and oviposition was observed inside the cage. After oviposition the pots were removed to the room for further study. The eggs were watched at regular intervals and thus incubation period was determined. After hatching, the first instar larva was studied. On the third day of hatching gall formation was seen. These galls were cut open at regular intervals and different instar larvae were obtained. In the same way many terminal tips of the plant were plucked and brought to the laboratory for further examination. The eggs thus obtained were kept in a cavity glass block with wet cotton wool inside desiccator. After hatching they were transferred to an artificial medium which was prepared by Agar Agar soaked with the extract of the plant. But due to immense growth of fungus, the experiment could not succeed. For the determination of the pupal period and to know their mode of life many fourth instar larvae were kept in the cavity-blocks which were placed inside the desiccators containing sufficient humidity in controlled temperature room. The following methods were used for morophological study of various larval and pupal stages.

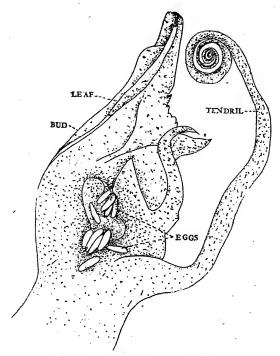
Eggs: The eggs were studied in salt solution and then mounted in glycerine for observation. For permanent mount, eggs were fixed in Carnoy's fluid for about two hours and then stained in Acid-fuchsin and mounted in Canada baslam.

Larva: The preserved material was used for whole mounts of the larvae, pupae and empty pupal cases. They were pricked with fine needle and boiled in 10% KOH until the body contents became soft. After squeezing the empty skin was treated with glacial-acetic acid for clearing. They were then transferred to a drop of clove oil and finally mounted in Canada-balsam.

The usual method of killing the larvae in hot water and fixing them in Bouin's fluid, as recommended by many workers, was also applied.

Gater's fluid was also used for the study of both external morphology and tracheal system. It was prepared according to the formula given in Bolles Lees' Vade Mecum.

A new mounting medium consisting of Polyvinyl alcohol has also been used with great success. In this medium the larvae and pupae were mounted directly from life or from preserved material without using the tedious time-consuming processes of fixation, dehydration and clearing. This mounting medium was prepared with Polyvinyl alcohol PB according to the formula described in detail in a previous publication of the author.¹ Eggs, larvae and pupae were mounted in this medium and photographs taken. The respiratoy system became very clear and it gave better results than Gater's fluid. A little heating made the preparation suitable for immediate examination under oil-immersion lens. Permanent slides were then made.



Eggs on the leaf-bud of Coccinia indica.

#### HABITAT

The plant Coccinia indica belongs to the Cucurbitaceae family and it is a perennial creeper with tuberous roots. Leaves are of 2-4 inches in diameter, five angled or lobed, cordate, shining, scabrid and petiolate. Flowers are white and fruits are avoid or oblong, bright-scarlet when ripe with seeds embedded in red pulp. This plant thrives well with all vegetative parts throughout the year. Due to the cold in the months of December and January, the leaves become yellowish-green with

Grover, P. (1962) A new mounting medium. National Academy of Sciences, India. Abstracts
of contributed papers.

emerginate margins. In the month of February the plant gives rise to many new growing shoots on the old stems. The new leaves are like those found in the rainy season and within a week's time the climber covers the big herbs and shrubs. The climber is common in India and is found everywhere on shrubs and even on big trees like mango, date, palm, etc.

#### GALLS ·

The midges produce gall on the stein, petiole and even on the tendrils of the plant in abundance after the rains and continue making galls upto the beginning of November. Dried old galls are easily visible during the month of December hanging from the shoots but some of the old greenish-yellow galls are also seen hidden below the growing plant.

The galls (Plate I, C) are regular, ovoid, fusiform or moniliform, solid and fleshy structures when young. As they grow old they become harder and harder. In size they vary from 2-9.5 mm. long and 2.5 to 4.0 mm. thick. They are darkgreen in colour when young and apple-green when old. The galls are glaborous (smooth) and when opend they show definite tunnels with hardened walls in mature galls. These walls have been designated as cysts; therefore, these galls have been called cystiferous. If the galls are kept for rearing, the covering epidermis thins down and the cysts become visible as nodules especially when the galls are wet. The galls are indehiscent, that is, they do not split or burst to allow the larvae to pass out. The larvae, on the other hand, bore their way upto the epidermis of the plant and pupate. The epidermis is punctured by the pupa. If the galls are attacked by hymenopterous parasites they show black streaks on the surface. When inquilines, like Dacus, lay eggs in the gall it attains enormous proportions. When the flies leave the gall it become straw-coloured and dries up. It shows a number of circular holes on the surface and from some of these holes empty pupal skins project (Plate I, D). The galls on the petioles are similar to the stem galls but are usually smaller and oval. On the leaves the galls are usually formed on the veins. Each gall contains many larval chambers or tunnels which are long and irregular in shape (Plate I, E). In the beginning the tunnels are in centre but in the advanced stage the tunnels are constructed below the epidermis for pupation.

Galls are formed due to some chemical reaction between the plant and the parasite. Usually the parenchyma cells proliferate to great extent under the influence of the parasite while the growth of the epidermal cells remains slow as compared to the former. The proliferating parenchyma, therefore assumes extensive proportions and is responsible for the characteristic bulge of the galls. Study of the transverse and longitudinal sections of the galls reveals this clearly. The conducting tissues, however, are not disturbed in the earlier galls and as such further growth of the shoot is not effected in the beginning. During winters the colour of the galls changes from green to brown and they become very hard.

There are many different views regarding the formation of galls which are beyond the scope of the present study; however, those that merit attention are briefly discussed. Malpighi was the first entomolgist who explained the cause of gall formation and according to him, the poisonous fluid of oviposition causes, irritation that results in swelling of the plant tissues enclosing the eggs. Alser (1877),\* Busgen (1891),\* Laboulbene (1892),\* Cook (1921),\* Rossing (1904),\* Cholodkovasky (1905),\* Borner (1908),\* Molliard (1913),\* Dewitz (1915),\* Nemec (1924),\*

and others were of the view that the gall formation is due to the active action of salivary secretion of the larvae but not because of toxic fluids released during oviposition. According to Zweigelt (1931)\* galls are the result of biological and morphological adaption not only on the part of the parasite but also on the part of the plant tissue. The larvae are only concerned with the stimulative phase of gall formation while shaping of the gall belongs to the domain of the plant. This process of mutual adaption is very slow and gradual. The galls not only provide nourishment to the larvae but also afford protection. Thus, this complicated structure of the plant provides much benefit to the parasite. It is, however, certain, as mentioned above, that it is the intimate association of the host and parasite that is responsible for gall formation. As the nourishment is drawn through the surface contact and excretory products are similarly passed on the plant tissues, their chemical reaction provokes the gall formation.

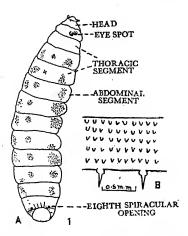
In addition to midges several other animals as well are associated with the gall both before and after the escape of the midge. They are:

- 1. Gall makers.
- 2. Inquilines such as Dipterous flies.
- 3. Parasites on the host and inquilines such as certain Hymenoptera.
- Commensals.
- 5. Successors such as thrips and bugs.
- 6. Predators on the host and inquilines such as certain Hymenopters.
- 7. Causal visitors as ants.
- 8. Heyper-parasites on parasites of the host, on commensals, successors, 8. Heyper-parasites on purchase etc.

Oviposition: B. toombii always prefers young green leaf buds, which she seems to locate with the help of the antennae. She then proceeds to them in a characteristic posture with the antennae arched and the tip of the abdomen bent forward inbetween the legs. On reaching the proper spot she inserts the ovipositor quickly between the overlapping leaves and lays eggs usually on the concavity of the leaves of the growing terminal bud (fig. 9) holding the bud firmly between the legs; all the time the wings are held vertically up in a ready-to-fly posture and butfor slight casual movements, sometimes, the antenae remain practically motionless. The whole act of oviposition lasts for three to four minutes, rarely longer. After laying the first batch of eggs the midge flies round the bud and with the help of the antennae locates another side suitable for oviposition. It may be either to the right or left of the original spot. In this way she lays eggs in five to six batches, each containing twelve to fourteen eggs. Then she leaves the bud and moves off slowly stretching her abdomen apparently toileting it with legs. After a while she may fly to a new bud or oviposit again on the same bud. On an average one female lays more than one hundred eggs. Curiously enough these midges never oviposit in capitivity. The eggs are usually laid in the afternoon,

<sup>\*</sup>Reference quoted from Mani (1948).

The frequency and duration of oviposition depends upon the suitability of the host, humidity and temperature of the atmosphere.



Text-figure 1A. First instar larva, 1B. body wall of the same under oil immersion.

#### CAGE OBSERVATION

In order to study the process of copulation and oviposition the flies, from the rearing cages, were transferred to the field-cage and their activities were watched. In the first instance the flies were released at about 7.30 a.m. and observed. Some of them were seen visiting the plants and only one pair was seen copulating; then the female visited some growing tips of the shoot and came to rest on one of the buds which had a small gall as a result of a previous infection. Visiting ants, however, disturbed the midge but she kept on hovering around the same shoot and attempted to alight on it at least four times. Finally, she laid eggs on the same tip, in the afternoon, between 3 to 3.20 p.m. The shoot was plucked and brought to the laboratory for further observations. Eggs were found in five to six groups each with twelve to fourteen eggs. Then they were kept in glass cavity-blocks with wet cotton in a desiccator for hatching.

Next time the flies were released in the field-cage at about 2 p.m. in the afternoon. At 3 p.m. one fly was seen laying eggs. As usual the fly changed her position six times and laid eggs in six batches. The oviposition was completed within a short period of twenty minutes ending at about 3.20 p.m. Afterwards the fly was disturbed while attempt was made to drive away an intruder (a spider). The fly did not visit the shoot again, but was seen resting on the wall of the cage for a long time when, with the approach of darkness, she was caught for microscopical examination.

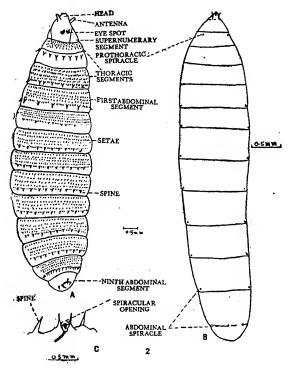
#### EGG

The eggs (Plate II, J) of B. toombii are minute, cylindrical and rounded at both ends the posterior end being broader than the anterior. Each egg is 0.30 mm long to 0.09 mm, wide. It is bright dark orange in colour and sticky in nature enclosed in a transparent shell below which lies the thin yet distinct vitelline membrane.

The incubation period of the egg varies from two to three days depending upon environmental conditions.

The larval phase is the longest and in Bimba toombii it is divided into four instars. The different larval instars are usually similar in form but differ in size and in certain anatomical details. According to some authors there are only three larval instars. Marchal has described three phases in the case of Gecidomiya destructor: (i) the freshly hatched larva; (ii) the phase of nutrition and growth; (iii) the quiescent and non-feeding stage before pupation. Some have not been able to confirm the actual number while Hamilton (1923) in Monarthropalpus buxi has described four instars. Matcalfe (1933) also found four instars in Dasyneura leguminicola Lint. The four instars of the present midge have been distinguished on the basis of size and of important morphological differences as will be evident from the account given below. It may, however, be added that like many other insects it is the larva that does the main damage.

Paedogenetic larvae are of common occurrence in cecidomyids, but the same have not been found in B. toombii.



Text-figure 2A. Second instar larva (dorsal view); 2B. spiracular opening of the second instar larva; 2C. prothoracic spiracle highly magnified.

#### First instar larva :

From the eggs hatch immature worm-like larvae. The freshly hatched larva (fig. 1A) is about 0.3 mm. long and 0.1 mm. wide. It is reddish-orange in colour and with distinct body segments. The head has a pair of antennae and is somewhat spatula-shaped, which is strengthened by a thin circular chitinous ring from within.

The mouth parts are well differentiated even in this instar. They have the same structural make-up as those of the fourth instar larva, but are not as much sclerotized. The body consists of head followed by the neck, three thoracic and nine abdominal segments. The head and the neck segments are retractile in the first throacic segment. As in other cecidomyid larvae the neck segment is provided with a pair of eye-spots which are yellowish-brown, somewhat crescentic in shape and are placed side by side. The ninth abdominal segment is small and very distinct. The body of larva is slightly flattened dorsoventrally and provided with minute spines and papillae which are only visible under oil immersion lens. In each segment there are four rows of small setae and one row of spines below the setae (Fig. 1B). Spines of the penulimate abdominal segment are much developed as compared to those of the rest of the segments.

The body is transparent with a deep-orange patch (embryonic yolk) in the posterior abdominal region. A pair of open spiracles on the eighth abdominal segment is very distinct. The larva shows certain masses of cells which appear opaque in the mounts. These masses have been referred to as fat bodies by many authors but they are actually masses of formative cells that from the organs of the imago as such they are called imaginal discs or imaginal buds (Plate II, H). They are constant in position and are relatively inconspicuous and grow in size in further larval instars as will be evident for the description that follows.

The larva is sluggish for the time being but soon becomes active and starts crawling on the veins of the leaf and even on the tendrils of the growing shoots. Finally it penetrates the tissues of the bud. Sometimes it may bore its way into the petiole or tendril.

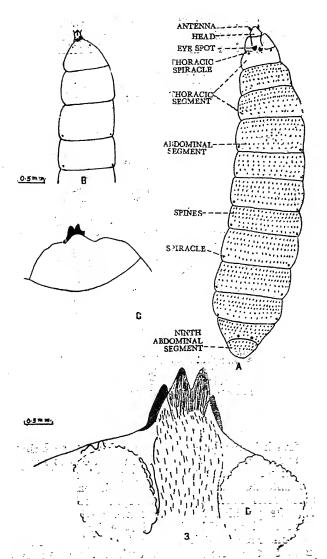
#### Second instar larva:

It is slightly more than 0.1 mm. wide and 0.3 mm. long and does not differ much in colour and size from the first instar larva except in the openings of the spiracles (fig. 2 A, B and C) which have increased. There is one pair on the prothorax and eight on the first eight abdominal segments. The prothoracic and the eighth abdominal spiracles open dorsally, whereas others on the dorso-lateral side. There is no change in the papillae and spines of the body. The larva moves away from the surface of the plant tissue and when it reaches almost the middle it induces the formation of the gall.

#### Third instar larva:

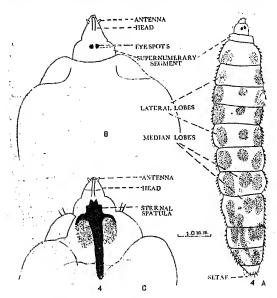
The third instar larva (fig. 3A) is very light-orange in colour and is about 2·1 mm. long and 0·4 mm, wide. The mouth parts of the larva are more differentiated and so is the case with tracheation, the number of spiracles remaining the same as in the second instar larva. The papillae on the body segment are more differentiated than those of the second instar and less conspicuous than those of the fourth instar larva. In the beginning, the larva is of light colour but it acquires a dark yellowish orange colour as it advances in age. The imaginal discs of this larva (Plate II, H) are also more developed than those of the previous stage. The characteristic chitinous structure, called sternal spatula, makes its first appearance on the ventral surface of the prothoracic segment of the larva in this stage only. The sternal spatula is characteristic of the gall-midge larvae and

<sup>1.</sup> Several other names have been given to this structure. The English authors have called it "anchor process" "anchor plate" or sternal spatula. German authors have used the words "brustlein" or "brustgrate". Osten Sacken calls it "Breastbone" Mik calls it "Spatula sternals" Marchal calls it "Spatula sternals" Marchal calls it "spatula".



Text-figure 3A. Dorsal view of the third instar larva.; 3B. ventral view of the same showing the beginning of the sternal spatula; 3C. ventral view of the sternal spatula enlarged; 3D. ventral view of the third instar larva showing sternal spatula.

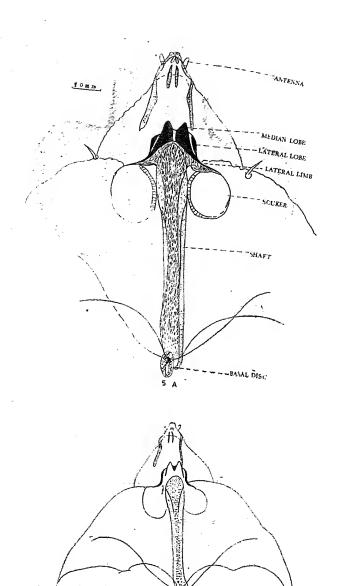
differs in shape in different species and genera (Kieffer 1900). The third instar larva can be differentiated from other instars by the presence of sternal spatula (fig. 3 B, C) which is not fully formed and weakly sclerotized. It is formed by a thickening along the median line of the ventral body wall. Normally the spatula consists of a shaft having quardridentate crown towards the anterior side. In the third instar larva the shaft (fig. 3 D) is not differentiated; only the crown is distinct with two central dents more sclerotised than those on the sides. Consequently, the central dents appear light in colour and those on the sides are creamy.



Text-figure 4A. Imaginal discs of the fourth instar larva; 4B. dorsal view of the anterior part of the fourth instar larva; 4C. ventral view of the anteror part of the fourth instar larva, showing the fully formed sternal spatula.

#### Fourth instar larva:

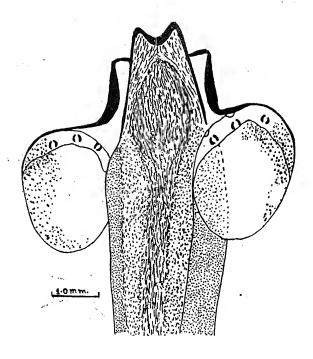
The size of the fourth instar larva shows a wide range being 3.2 mm. long and 0.7 mm. wide on average. The mature larva (fig. 4A) is more easily reocgnised by its deep yellowish orange colour, by prominent imaginal discs and distinct bi-segmented antennae, each with a conspicuous basal sclerite. The mouth parts are more sclerotised and the eye-spots are blackish in colour and much differentiated. The papillae and spines are well developed and heavily sclerotized. As in the second and third instar larvae there are nine pairs of open spiracles, one on the prothorax and eight on the first eight abdominal segments. The spiracles (fig. 2 C) of the first seven abdominal segments open on the dorsolateral region. Whereas, the first pair of prothoracic and eighth abdominal spiracles open on dorsal side of the body slightly away from the lateral line. The sternal spatula (fig. 5 A-E and Plate II, K) is fully developed and yellowish brown in colour. Posteriorly the shaft is lodged in a pocket shaped invagination of the body wall. The sides of the crown are drawn out into two posteriorly curved processes embracing two rounded sucker-like muscular structures which extend posteriorly as much as half the length of the shaft. When fully formed the sternum

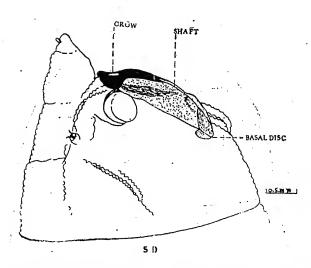


l'ext-figure 5A. Enlarged diagram of fully developped sternal spatula;
5B. sternal spatula showing the folds at the base and also the basal disc.

5 B

0 5 mm.





Text-figure 5C. Anterior portion of the sternal spatula showing highly magnified suckers; 5D. Side view of the anterior part of the fourth instar larva showing the fully former starnal spatula.

is a very prominent structure of the larva. On observing a locomoting larva the dark-brown dents of the crown are visible even from the dorsal side when the head is retracted within the anterior segments of the body. The movements of the sternum, it seems, are controlled by the posterior pocket and the lateral sucker-like structures. As the procket straightens it pushes the sternum anteriorly and as the body is pulled forward the suckers hold it at the spot. Thus forward and backward movements of the sternum seem to aid in cutting the plant tissues.

Different authors have ascribed diverse functions to the sternal spatula of the larva. Curtis (1845)\* studied it in the Hessian fly Mayetiola destructor and ascribed a locomotory function to it. According to Enoch (1891)\* it is used for reversing the position. Giard (1893)\* suggests that it is responsible for leaping movements, Sharp (1918) agress with him. Williston (1908) and Comstock (1930) have not been able to determine its function while Metcalfe (1933) agress with Enoch. Sen (1938) considered it to be useful in abrading the plant tissue in Rhabdophaga salici perda.

In the light of the present work this seems to be a boring structure that also helps in the ecdysis of the late fourth instar larva into pupa. As the fourth instar larva withdraws the head quickly within the trunk the dorsally-turned dents of the sternum rub against the ventrally turned fold of the skin above. In the prepupation larva the head is retracted numerous times in quick succession. The point of skin that is thus rubbed by the dents is the place where the larval exuvium opens to allow the emergence of the pupa. As the pupa wriggles out the exuvium moves behind and remains attached to the posterior end of the abdomen sometimes for quite a time so much so that to note the sex of the advanced pupa the exuvium has to be removed. That it helps in boring is further suggested by the fact that in the larvae, that pupate in the sand, the crown is not dentate or, if at all dentate, their number is less, usually two, and they are less chitinized. In Aschistonyx baranii that pupate in sand, the crown has a rounded margin in front instead of dents. The larva of B. toombii is gall inhabiting and as such it has to move within hard tunnels and usually to bore within soft tissue. Before pupation the larva cuts the tissues of the gall upto the epidermis. Actual perforation of the surface is not done by the larva but by the pupa before emergence. Naturally the four dents of the crown help the larva in making tunnels and in moving within them. In another gall-inhabiting species, Lasioptra asterspinosae White, the sternum has a flattened shaft and a tridentate crown with side-processes. Naturally, the dents seems to be associated with cutting the tissues inside the galls.

The view that sternal spatula is used to cut the plant tissues finds its full support from Felt who calls it a 'boring tool'. As the cutting is done by scrapping the views of Ormerod (1886)\* seem to agree. I do not very much agree to use the word perforation since it is not the larva that cuts the holes (perforation), as mentioned above, but it is the pupa that perforates the epidermis. I, however, find myself in full agreement with Sen (1938) when he says that the use of the sternal spatula, as a locomatory organ, is "highly improbable", as in this case also the movement of the larva takes place by rhythmic contraction and expansion of the body.

The imaginal discs and fat bodies are very conspicuous in this stage. There is a pair of imaginal discs in the dorso-lateral region of all the thoracic and abdominal segments and one lobe in the middle of each segment. Similarly, there is

<sup>\*</sup>References as quoted from Sen, P., (1939).

a series of ventral lobes arranged segmentally along the median line. This arrangement of imaginal discs is almost similar to that found in many other species such as Cecidomyia destructor Marchal (1897), Dasyneura leguminicola Metcalfe (1933) and D. lini Pruthi (1937).

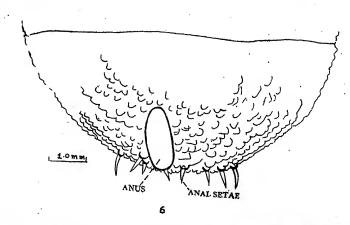


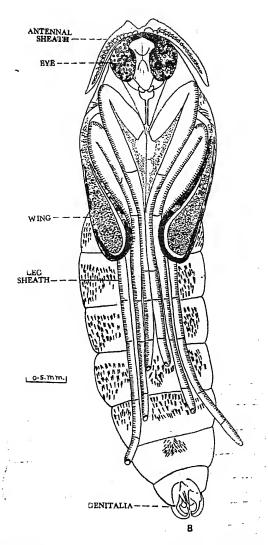
Fig. 6. Posterior part of the fourth instal larva to show the anus and anal setae.

The anus is situated ventrally and anal segment bears four pairs of short setae located on tubercles (fig. 6, Plate II, L).

The fourth instar larva in this case constitutes the overwintering stage. Ordinarily, the fourth instar lasts for about three to four days but, with the approach of winter, their pupation is delayed so much so that they remain in that stage for the whole winter and pupate only on the return of normal conditions towards late June or early July. To study this in greater detail the galls, containing fourth instar larvae were kept in the laboratory and were opened from time to time. They always showed healthy fourth instar larvae. Some larvae were taken out in the month of November and kept in glass cavity blocks at room temperature. They continued to grow normally although appeared to be sickly. In the month of January some of these larvae were transferred to constant temperature room having a temperature of 30°C and were kept inside in desiccators containing higher humidity of 60%. Under these artificial conditions the climatic diapuase of the larvae was broken and they pupated finally leading to normal emergence.

#### PUPATION

When the fourth instar larva is ready to enter the pupation stage it makes its way towards the epidermis of the gall and becomes practically motionless before pupation. The head and the terminal abdominal segments are retracted. Before the larval skin is actually cast off the larva shows a sudden rhythmic body movement of contraction and retraction and the skin is cast off. The sternal spatula is also shed off with the larval skin and is seen attached to the posterior tip of the pupa. Before emergence the pupa forces its way out of the epidermis of the gall and when the upper half of the pupa projects from the surface eclosion of the imago takes place. The pupation period is normally of six days duration but later on it is nearly 13 to 14 days due to change in the temperature. There is no cocoon formation. It is interesting to note that under laboratory conditions the pupation time is the same as that of the natural conditions, and pupae thus formed are apparently quite normal and give rise to normal flies.

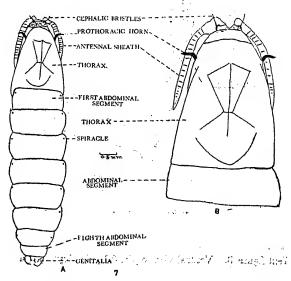


Text-figure 8. Ventral view of the fully developed pupa.

Following the moult of the last larval instar the insect transforms into the pupa (fig. 7 & 8; plate II, G). The pupa is naked, 3.3 mm. long and 0.8 mm. wide and the distinct divisions of the head, thorax and abdomen are visible. The colour of the pupa, soon after ecdysis, is light yellow which soon takes up an orange shade finally becoming blackish-brown dorsally and lighter ventrally. The following is the detailed description of the pupa:

Head: The head is very well differentiated and bears all the characteristic structures such as the antennae, eyes and the mouth parts. But all these structures are transparent. There is a hair-like structure behind each eye and a pair of spines on the apex of the head. Examined after twenty four hours the pupa appears orange in colour and the external structures become evident. Ommatidia can be distinguished as minute dots while the antennae and the mouth parts also get differentiated. On the third day, the eyes appear dark red, the antennae brownish and so is the case with the mouth parts. All these changes are related with chitinisation. Finally, by the time the pupa is ready for molting into the adult the eyes become black in colour and the antennae and mouth parts become dark brown.

Thorax: In the newly formed pupa the thorax bears developing wing pads dorsally and three pairs of delicate legs running along the ventral wall of the abdomen reaching upto its middle. The wing pads start showing nervures in a pupa of about 48 hours and by the end of the third day the venation becomes distinct. Before the emergence of the imago the hyaline wings are clearly visible through the transparent covering. The legs become more and more brown their joints become clear and they become longer reaching the tip of the abdomen. The thorax also carries two spiracles located on the tip of two horn-like processes one on each side of the prothorax.



Text-figure 7A. Dorsal view of the pupa; 7B. enlarged antreior portion of the pupa (dorsal view).

Abdomen: The abdomen also appears transparent in the beginning but with brisk chitinisation it turns brown. The dorsal surface of each segment becomes darker than the ventral apparently because more chitin is deposited dorsally than on the ventral side. Towards the end of the pupal instar the abdominal segments show whorls of scales running in the middle of all the segments. In the beginning the abdomen of both the male and female pupae is similar but towards the end of the pupal phase the genitalia differentiate. With the help of gentitalia the male and the female pupae can be recognised.

Movements: The pupa of B. toombii is capable of slight movements. Normally, it keeps on lying on the back and growing but sometimes with the help of the leg and contraction and expansion of the abdomen it can move about. When disturbed with needle they always move and change position. Within the galls, however, it is likely that they may remain inactive till the last phase of the pupal instar in which they become suddenly active. The abdomen shows characteristic rhythmic contractions and expansions pushing the body this way or that. These movements are also accompanied by the movements of the legs. In this stage the pupa moves up in the tunnel in which it lives and comes close to the epidermis and finally perforates it. Through this the pupa pushes out its anterior part and projects from the surface till the fly emerges. The rhythmic movements of the body continue further and in all probability aid the insect in puncturing the puparium for emergence. To study the movements the pupae were kept in cavity blocks and watched under binocular microscope. In the perforation movements the pupae only wriggled forward but in the pre-emergence movements the pupae raised the abdomen up keeping attached to the surface by the head and all the time the abdomen continued contracting and expanding. Then suddenly the pupa would attach its tail end to the surface and would raise the head up. headon-tail and vice versa movement seems to be the result of exaggerated rhythmic activity directed to burst open the puparium. As the pupae in the cavity blocks lie without any hold around them the force of the contraction and expansion turns them upside down or vice versa. But within the galls they are firmly held by the tunnels through which the anterior end projects out and as such the entire force of the rhythmic body movements is directed against the puparium which soon gives way allowing the emergence of the imago.

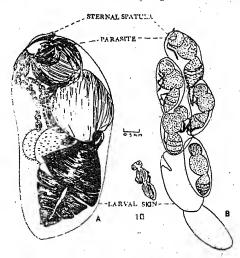
#### **GENERATIONS**

All attempts to ascertain the period of different larval instars failed. But as fresh batches of eggs on the growing tips have been observed every now and then and fresh galls kept on appearing throughout the period extending from July to November it may be concluded that there are several generations in a year. The length of the last generation involving the overwintering fourth instar larva is too long.

#### PARASITISM

The larvae of B. toombii are sometimes found infested with a number of hymenopterous parasites. It seems likely that only the third instar larva is parasitised, for the earlier stages do not show any effect of parasitism. In the early stages of parasitism it is difficult to distinguish between a healthy and a parasitised larva but as the development advances external signs of parasitism become visible. The parasitised body of the larva becomes elongated and it becomes sluggish and segmented. The imaginal discs and fat of the larva decrease and soon the growing parasite becomes conspicuous in the form of a chain lying within the larva which by now is practically consumed. To allow the parasite to complete its life-cycle

the larval life is lengthened and may be for this reason that the internal organs of the larva, except the fat bodies, are not affected for a long time. The adult parasites pierce through the body wall of the larva and finally emerge from the gall through minute holes formed at the time of egg laying by the female parasite. The life cycle is completed inside the gall along with that of the gall midges.



Text-figure 10-A, Hymenopterous rarasite Mesocylops within the larval skin of the host; B, chains of embryos of the parasite.

Chains of one type of hymonopteruos parasite embryos have been found (fig. 10; Plate II, J), belonging to the genus Mesocyclops of the family Proctotrupoidae. Towards the end of autumn the parasitisation is very heavy so much so that nothing but the parasites emerge from the galls.

#### PARASITES AND INQUILINES

Associated with the galls of B. toombii a number of other hymenopterous and dipterous insects have also been noted. They have been reared along with the midges in the laboratory. These include Terastius species (Chalcididae). Dupolmus sp. (Eupelmidae), Brocon species (Ichneumonoidae) among the Hymenoptera and Desmometopa palpalis de Meij Atherigona orientalis Salime, Leptocera crassimana var. Clunipes Meigen, Conicera (species not known) and Dacus cucurbitae Coq. among the Diptera; of these Decus cucurbitae has been studied more carefully. It does not seem to be a parasite of the midge but just an inquiline.

From a number of galls white spindle-shaped eggs of *D. cucurbitae* have been recovered. Different larval stages were not uncommon. The eggs were seen within young galls in large number and larvae in the decaying galls. The jumping larvae are of yellow colour and usually jump out of the walls of the rearing chambers. Some other dipterous larvae behave in the same way.

#### ACKNOWLEDGEMENT

The author is indebted to Dr. M. D. L. Srivastava, Professor and Head of the Zoology Department, University of Allahabad, for affording facilities for the work and to the Government of India for awarding Research Fellowship which enabled the author to undertake the study. She wishes to express her gratitude to Dr. S. N. Prasad for constant guidance and supervision. Lastly the author thanks

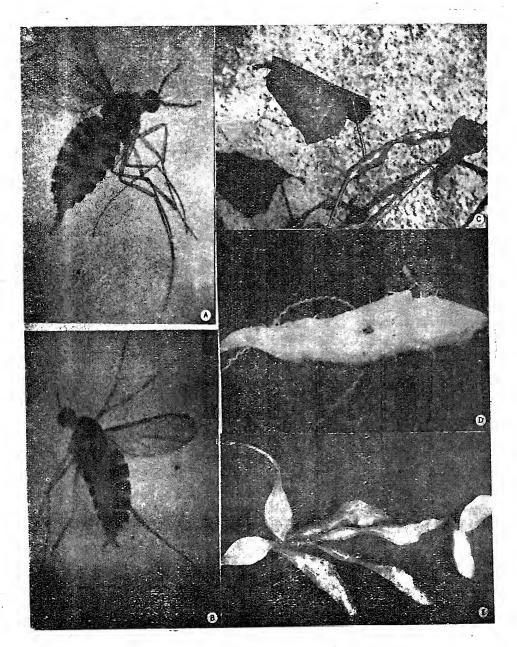
Mr. J. P. Doncaster, Keeper of Entomolgy, British Museum (Natural History) for , getting the hymenopterous parasites, reared from the galls, identified.

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PLATE'I

- A. B. toombii, adult female.
  B. B. toombii, adult male.
  C. A portion of the plant showing stem galls.
- D. Oldigall showing pupal exuviae projecting from the surface.
   E. Gall cut open to show larval tunnels in the interior.

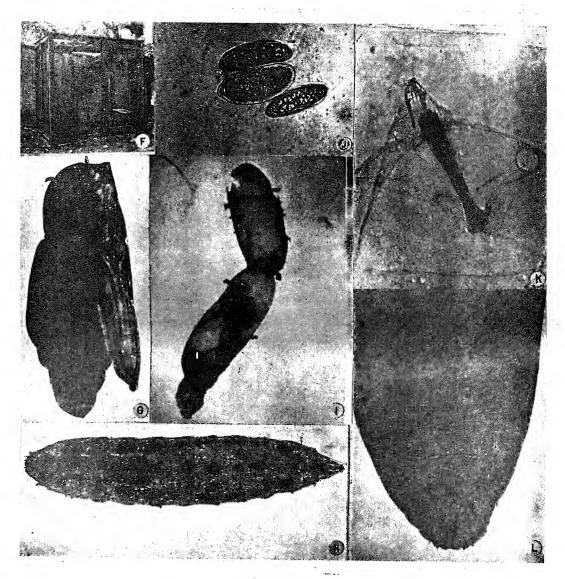


PLATE II

- F. Photograph of the field cage (6×6×3) erected to study copulation, legg laying and gall formation, etc.
  G. Microphotograph of the pupa of the midge (10×10).
  H. Microphotograph of the larva of the midge showing imaginal discs. (10×10).

- I. Parasitic Hymenoptera inside the larva of
- I. Parasitic Hymenoptera inside the larva of the midge.
  J. Microphotograph of the eggs of the fly (10×10).
  K. Microphotograph of anterior part of the fourth instar larva (10×10).
  L. Microphotograph of the posterior part of the fourth instar larva (10×10).

#### ON THE QUESTION OF TRANSMISSIBILITY OF FLOWERING STIMULUS RESULTING FROM VERNALIZATION IN INDIAN CROP PLANTS

Вy

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That the flowering stimulus caused by photoperiodic induction could be translocated through graft union is now a well established fact. As regards vernalization, the first successful experiments are of Melchers (1939) where he recorded that if cold treated individuals of biennial forms of Hyoscyamus niger are grafted to non-cold treated ones, the latter are induced to form flowers. A few more cases of similar flower-inducing grafts have been mentioned by Lang (1952) and also by Chouard (1960). Several experiments along these lines have also been reported by Japanese workers in radish and cabbage (Kojima et al, 1953, Kagawa, 1957 and Tashima, 1957). Physical existence of a flower forming substance due to vernalization treatment has been demonstrated by Purvis and Gregory (1953) where they recorded a significant reduction in the scores of winter rye treated with chloroform extract of the vernalized seeds. Tomita (1956) obtained acceleration of heading in winter wheat sown in spring raised from seeds soaked in diffusate of vernalized rye seeds in de-ionized sterile water. He (1957) also recorded an early heading in spring wheat treated with ether soluble part of the diffusate obtained from vernalized winter wheat.

A series of grafting and extraction experiments according to the techniques detailed below were undertaken by the author with Brassica campestris L., Lens esculanta Moench and Cicer arietinum L. to confirm the above findings.

#### Grafting:

Vernalized and control plants of Mustard T. 10 S. 13 were planted side by side in pots at Agra during the year 1950 and they were approach-grafted at the various stages of growth. Neither any reduction in the node number nor any earliness in flowering were obtained in non-vernalized plants though there was an earliness of 30 days in the vernalized ones. Later on at Bhopal during the years 1955-57 a series of side and cleft-grafting with seedlings about 7 to 10 days old were undertaken along the lines of Kojima et al but no positive evidence of the translocation of the vernalization effect could be obtained.

With Cieer approach grafting was carried out with vernalized and control sprouted embryos separated from the cotyledons and also cleft-grafting with the plumule tips of the vernalized ones as scion and control ones, decapitated just above the cotyledonary nodes, as the stock. By the time tissue union was complete buds at the axil of the cotyledonary nodes sprouted leading to the development of the axillary shoots. A record of flowering of these side shoots were kept with the presumption that if substance or substances formed during the process of vernalization is translocated through the graft union, then it must bring about an earliness in the flowering of the side shoots, which however, did not prove to be true.

#### Extraction:

This was tried both with distilled water, organic solvents and agar agar. Nearly 200 maximally vernalized seedlings of Brassica and Lens separated from their cotyledons both fresh from the refrigerator and air dried were taken for each solvent. Fresh embryos were pounded with sand and transferred to a 50 cc. flask containing about 20 cc. of chloroform, normal butyl alcohol, tertiary butyl alcohol, acetone, benzene, ethyl alcohol, carbon disulfide, petroleum ether, ethyl ether, xylol and distilled water and allowed to stand both at the room temperature and also in the refrigerator maintained at 4-6°C. with occassional stirring for about 48 hours. The mixture was then centrifuged and decanted and the solvent evaporated under reduced pressure. Residue thus obtained was treated with 10 cc. of distilled water and the resulting solution centrifuged. Clear liquid thus obtained was used for soaking 10 seeds of the type from which the extraction was made till their testas were broken. These were then sown along with untreated seeds, brought upto the same state of physical development, in flower pots and time taken for anthesis of the plants raised from them recorded. These experiments were repeated several times under the climatic conditions of Agra and Bhopal with negative results.

For agar agar extraction, about 100 sprouted vernalized plumules without cotyledors of Brassica and Lens were washed with sterile water and transferred into sterile Petrie dishes over which a 2% agar agar solution was poured to fill in about 1/3rd the height of the dish. After 8 hours, 10 seeds of the same type washed with sterile distilled water were placed on the top of the agar agar block and allowed to sprout. These seeds were then sown along with their corresponding control in flower pots. Out of about a dozen experiments carried out only in one sowing (October, 1951) a significant earliness of 5 days was obtained with Lens, which, however, could not be confirmed later on.

The author agrees with Purvis and Gregory (1953) that negative results do not disprove the existence of substance or substances formed during low temperature treatment of seeds that leads to early flowering in plants raised from them.

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# ON TWO NEW SPECIES OF THE GENUS TREMIORCHIS MEHRA AND NEGI, 1926. (TREMATODA: PLAGIORCHIDAE)

By

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#### INTRODUCTION

Mehra and Negi (1926) established the genus, Tremiorchis for the reception of their species Tremiorchis ranarum recovered from the small intestine of Rana tigrina. Bhalerao (1926) described another form, Centrovitus pentadelphi from the intestine of Rana tigrina. Verma (1930) described T. varanum from the small intestine of Varanus bengalensis and V. grisens from Allahabad. In this paper he synonymised the genus Centrovitus Bhalerao, 1926 with Tremiorchis Mehra and Negi, 1926, and his species C. pentadelphi with T. ranarum on the basis of priority.

#### Tremiorchis mehrai n.sp.

Twelve parasites of this species were collected from the intestine of the common Indian toad, Bufo melanisticus Schneid, from Ghamapur locality in Jabalpur. The body spines are directed posteriorly and in the living condition they are seen to extend from the anterior end of the body to a little behind the posterior margin of posterior testis. They measure 0.017-0.024 mm. in length and 0.003 mm. in breadth at the base. The body, in eight mounted specimens, measures 5.25-5.79 mm. in length and 1.37-1.48 mm. in maximum breadth at the post-ovarian region.

The oral sucker is terminal and is always bigger than the ventral sucker. It measures 0.268-0.299 mm, in length and 0.268-0.331 mm, in breadth. The ventral sucker lies at a distance of 1.12-1.58 mm, from the anterior end of the body and measures 0.236-0.268 mm, in length and 0.236-0.252 mm, in breadth. The prepharynx is very small and leads into a muscular pharynx which measures 0.157-0.189 mm, in length and 0.178-0.189 mm, in breadth. The oesophagus measures 0.473-0.867 mm, in length and 0.063-0.094 mm, in breadth. The intestinal bifurcation lies at a distance of 0.931-1.23 mm, from the anterior end of the body. The caeca end a little above the equator of the body and their posterior tips end in front of the anterior testis.

The excretory system is 'Y' shaped, with a long stem and small cornuae. The stem of the excretory bladder extends to the posterior border of the ovary. From each cornua arises a common excretory duct, which further divides into two factors, one going towards the anterior end and the other towards the posterior side. The excretory pore lies at the posterior end of the body.

The testes are oval to spherical in shape. The anterior testis measures 0.394–0.631 mm. in length and 0.411–0.71 mm. in breadth and lies just behind the equator of the body. The posterior testis is obliquely situated behind the anterior testis and the distance between the two testes measures 0.031–0.1 mm. The posterior testis measures 0.394–0.741 mm. in length and 0.411–0.639 mm, in breadth. The vasa efferentia, arising from the anterior face of each testis, meet to form a vas deferens, which opens into the cirrus sac. The cirrus sac is curved and overlaps

a part of the ventral sucker; it measures 0.473-0.583 mm. in length and 0.078-0.236 mm. in breadth. It is situated on the right side of the ventral sucker and contain a small tubular vesicula seminis, a tubular pars-prostatica and a long tubular cirrus. The genital pore lies just in front of the ventral sucker.

The ovary is oval to spherical, submedian in position, and generally lying on the right side of the median line of body. It measures 0.331-0.363 mm. in length and 0.299-0.411 mm. in breadth. The oviduct, arising from the posterior side of the ovary, runs posteriorly to meet the duct of the receptaculum seminis. Laurer's canal is present and appears to be a posterior elongation of the receptaculum seminis. The receptaculum seminis measures 0.175-0.252 mm. in length and 0.315-0.441 mm. in breadth. The oviduct is then joined by a duct from the vitelline reservoir to form the Mehlis' gland. The vitelline follicles are arranged in groups of 3 to 8 cells and they extend from the anterior level of ventral sucker to the level of the equator of anterior testis. The transverse vitelline ducts join to form a vitelline reservoir. The vitelline reservoir measures 0.041-0.045 by 0.13-0.14 mm. in size.

The uterus has descending and ascending coils, contains numerous eggs, and extends a little above the posterior margin of the body. The eggs are operculate and measure 0.028-0.031 mm. in length and 0.008-0.014 mm. in breadth.

Host: Bufo melanostictus Schneid.

Habitat: Small intestine.

REMARKS Locality: Ghamapur, Jabalpur (M. P.).

Among the only two previously described species T. ranarum Mehra and Negi, 1926, (Syn. Centrovitus pentadelphi Bhalerao, 1926) and T. varanum Verma, 1930, the new species Tremiorchis mehrai resembles Tremiorchis varanum Verma, 1930, in the size of the suckers, i.e., the oral sucker being always bigger than the ventral sucker. The new species, however, differs from T. varanum Verma, 1930, in the extension of intestinal caeca, position of testes, position of ovary, extension and nature of vitelline follicles, smaller size of its egg and its larger body size.

#### Tremiorchis vitelloconfluentum n.sp.

Three specimens of this species were collected from the small intestine of the common Indian Frog, Rana tigrina Daud, brought from Piparia, 111 miles from Jabalpur.

The boby of the worm is covered densely with small cuticular spines, directed posteriorly, up to three fourth part of the body. The spines are triangular in shape. The body measures from 2.398-4.05 mm. in length and 0.656-1 mm. in breadth, the maximum breadth being at the acetabular region.

The oral sucker is terminal and measures 0.147-0.157 mm. in length and 0.15-0.173 mm. in breadth. The ventral sucker is bigger than the oral sucker, it lies at a distance of 0.741-0.931 mm. from the anterior end of the body and measures 0·171-0·252 mm. in length and 0·189-0·268 mm. in breadth. The mouth leads into a small prepharynx (observed only in the living condition), which opens into a well developed pharynx, the latter measuring 0·102-0·11 mm. in size. The pharynx leads into a moderately developed oesophagus, 0.331-0.347 mm. in length and 0.055-0.094 mm. in breadth. The intestinal bifurcation lies at a distance of 0.71 mm. from the anterior end of the body. The intestinal caeca end blindly,

slightly above or at the level of the middle of the body and their posterior tips are pretesticular.

The excretory bladder is 'Y' shaped, with a long stem and small cornuae. The stem of the excretory bladder extends to the posterior end of the anterior testis and then divides into two cornua. From each cornua arises a common duct, which divides into two factors. These two excretory factors run in opposite directions, viz., the anterior and the posterior, and each receives the collecting tubes of its side. The excretory pore lies at the posterior end of the body.

The testes are oval in shape, lying obliquely one behind the other and are situated in the posterior half of the body. The anterior testis measures 0.252-0.331 mm. in length and 0.173-0.236 mm. in breadth. The posterior testis lies at a distance of 0.015-1.4 mm. from the posterior margin of anterior testis, and measures 0.22-0.236 mm. in length and 0.236-0.362 mm. in breadth. The cirrus sac is large, curved and has thick muscular walls. It lies on the right side of the ventral sucker and measures 0.284-0.473 mm. in length and 0.071-0.102 mm. in breadth. It contains an elongated vesicula seminis, measuring 0.122-0.168 mm. in length and 0.035-0.038 mm. in breadth, a tubular pars-prostatica and a long tubular cirrus. The genital pore lies in front of the ventral sucker.

The ovary is oval, median, and lying at a distance of 0.014-0.015 mm. from the posterior end of the ventral sucker. It measures 0.181-0.236 mm. in length and 0.173-0.22 mm. in breadth. The oviduct arises from the posterior end of the ovary, runs posteriorly and joins the duct of the receptaculum seminis. The receptaculum seminis is quite small. Laurer's canal is not observed.

The vitelline follicles extend anteriorly to the middle of the ventral sucker and posteriorly to the anterior level of the anterior testis. The remarkable features of the vitelline follicles noted in this species are that they are not arranged in groups as described by Mehra and Negi, (1926), in Tremiorchis ranarum and are confluent in the ovarian region (Fig. 2). Due to this confluent nature of the vitelline follicles the species is named, Tremiorchis vitelloconfluentum. The follicles of the two sides are joined through their respective vitelline ducts to form a common vitelline reservoir, which then gives rise to a duct extending anteriorly and joining the oviduct to form the ootype.

The uterus is densely coiled and there are descending and ascending coils of uterus; its posterior limit reaching up to the posterior extremity of the body. The eggs are operculate, yellowish in colour, and measure 0.028-0.035 mm. in length and 0.007-0.01 mm. in breadth.

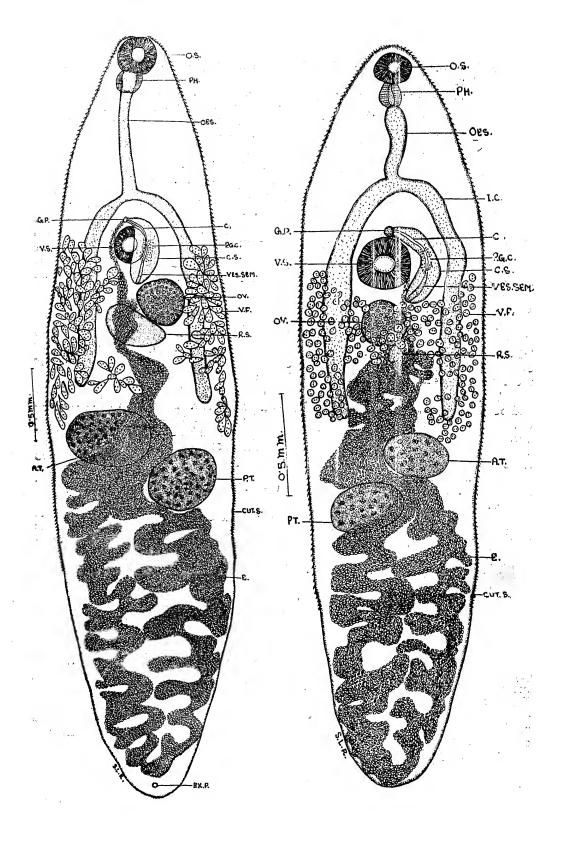
Host: Rana tigrina Daud.

Habitat: Small intestine.

Locality: Piparia, 111 miles from Jabalpur (M.P.)

#### REMARKS

Tremiorchis vitelloconfluentum n.sp. resembles T. ranarum Mehra and Negi, 1926, (Syn. Gentrovitus pentadelphi Bhalerao, 1926) in the size of its suckers (the ventral sucker being larger than the oral sucker). However, the new species differs from T. ranarum Mehra and Negi, 1926, (Syn. Gentrovitus pentadelphi Bhalerao, 1926) in its general spination, extension of excretory bladder, extension and nature of the vitelline follicles. The new species differs from all the species of the genus in its confluent nature of the vitelline follicles in the ovarian region, which is considered here to be of specific importance.



#### **LETTERINGS**

A. T.—Anterior testis; C.—Cirrus; C. S.—Cirrus sac.; CU.T.—Cuticular spine; E.—Egg; G. P.—Genital pore; I. C.—Intestinal caeca; OES.—Oesophagus; O. S.—Oral sucker; OV.—Ovary; PH.—Pharynx; P. G. C.—Prostatic gland cell; P. T.—Posterior testis; R. S.—Receptaculum seminis; V. F.—Vitelline follicles; VES. SEM.—Vesicula seminis; V. S.—Ventral sucker.

#### TABLE

### Showing the comparison of the two new species

	******	T. mehrai	T. vitelloconfluentum
1.	Study based on	12 Parasites.	3 Parasites.
2.	Host	Bufo melanostictus Schneid.	Rana tigrina Daud.
3.	Locality	Ghamapur, Jabalpur.	Piparia, 111 miles from Jabalpur.
4.	Extension of Body spines	Extend from the anterior end of the body to 2/3 of the body.	Extend from the anterior end of the body to 3/4 of the body.
5.	Body size	$5.25-5.79 \times 1.37-1.48$ mm.	2·3·8-4·05×0·656-1 mm.
6.	Oral sucker	Oral sucker larger than the ventral sucker.	Oral sucker smaller than the ventral sucker.
7.	Prepharynx	Very small.	Small,
8.	Pharynx	0·157–0·189 × 0·178–0·189 mm.	0·102–0·11 × 0·055–0·094 mm.
9,	Oesophagus	$0.473-0.867 \times 0.063-0.09$ rmm.	$0.331-0.347 \times 0.055-0.091$ mm.
10.	Shape and position of ovary	Oval to spherical, sub- median.	Oval, median.
11.	Nature and extension of vitelline follicles	Follicles arranged in groups; extends from the anterior level of ventral sucker to the level of the equator of the anterior testis.	Follicles not arranged in groups; extends from the middle of the ventral sucker to the anterior level of the anterior testis.
12.	Extension of excretory bladder	Stem of excretory bla- dder extends to the posterior border of the ovary.	Stem of excretory blad- der extends to the posterior end of anter- ior testis.

The two new species, T. mehrai and T. vitelloconfluentum differ from one another in the size of their body, general body spination, size of the suckers, position of ovary, extension and nature of vitelline follicles, and in the extension of excretory bladder and justifies their creation.

#### KEY TO THE VALID SPECIES OF THE GENUS TREMIORCHIS MEHRA AND NEGI, 1926.

- I. Oral sucker smaller than the ventral sucker.
- II. Oral sucker larger than the ventral sucker.
  - (1) Vitelline follicles extend from middle of ventral sucker to the anterior level of anterior testis; follicles not arranged in distinct groups; follicles confluent in the ovarian region. T. vitello confluentum n.sp.
    - (2) Vitelline follicles extend from intestinal bifurcation to the ends of intestinal caeca, arranged in groups, and not confluent in the ovarian region. T. ranarum Mehra and Negi, 1926. (Syn. Centrovitus pentadelphi Bhalerao, 1926.)
  - II., (3) Vitelline follicles extend from the level of intestinal bifurcation to ends of intestinal caeca, follicles not arranged in groups, and not confluent. Testes oblique. T. varanum Verma, 1930.
- (4) Vitelline follicles extend from anterior level of ventral sucker to middle of anterior testis; follicles arranged in groups, follicles not confluent. Testes oblique. T. mehrai n.sp

#### ACKNOWLEDGMENT . .

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#### A NOTE ON SOIL REACTION IN RELATION TO FUSARIUM WILT OF GRAM (CICER ARIETINUM L.)

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[Received on 7th June, 1962]

Wilt of gram (Cicer arietinum Linn.) caused by Fusarium orthoceras App. and Wr. var. ciceri Padwick, has been studied in relation to various ranges of soil pH (4.5, 6.0, 7.1, 7.8 and 9.2), in sterilized and unsterilized garden soils in pot-culture experiments. Twelve replications were maintained for each treatment, both for the experimental set and the control. The soil of each pot in experimental series was infested uniformly with the pathogen and for control, left uninoculated. Ten seeds were sown in each pot. The pH levels were adjusted as suggested by Ling (1941). The pots were kept in a glass house.

When the wilting started the mortality was recorded at regular intervals for each treatment in each pot separately. However, no plant wilted in the control. The figures for total mortality were analysed statistically. The effect of pH, individually and the interaction between soil condition (sterilized and unsterilized) and soil reaction has been found to be significant, which, is indicated in the table below:

TABLE Interaction between soil reaction and soil condition

(Figures relate to average percentage mortality for each pH level)

Soil ——Condition		pH ranges				Mean of all pH
	4.5	6.0	7·1	7.8	9.2	levels
Sterilized	35.75	59.59	35.75	19.06	11;91	32.41
 Unsterilized	9.53	13.10	32.17	34.55	55.12	28.89
Mean of soil conditions	22.64	36·34	33.94	26.82	33.51	<del>"</del>

C.D. at 5% level: Interaction 12.604: Soil reaction 8.900.

The mortality of plants was found to be influenced by soil reaction (pH), irrespective of soil condition. Different rates of mortality occured under sterilized and unsterilized soil conditions in various pH ranges. In sterilized soils the mortality was low in alkaline condition (pH 7.8 and 9.2) and in acidic ranges it was either equal to the neutral range (pH 7 1) or even higher. On the other hand under unsterilized soil condition mortality was high in the alkaline range and diminished considerably in acidic range.

Broadfoot (1933), Sanford (1941), Robert (1943), Ludwing and Henery (1943) and Subramanian (1946 and 1950), studied various factors regarding growth and pathogenicity of different fungi in relation to sterilized and unsterilized soil, and have shown that these conditions play an important role. Subramanian (1946) showed that 3% manure and 1% calcium monobasic phosphate had just the opposite effect on Fusarium vasinfectum, cotton wilt fungus, in sterilized and unsterilized soils respectively, which he attributed to the micro-flora present in the unsterilized soil. Almost similar findings are obtained here; the effect of pH on the incidence of the wilt disease of gram is altered if the soil is sterilized. In sterilized soil acidic pH is favourable to the disease development, while in alkaline pH it is suppressed, In sand culture experiments too acidic levels have been found to augment disease development, Chauhan (1962).

#### ACKNOWLEDGMENT

The author is grateful to Prof. S. Sinha for his guidance and criticism.

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## TWO NEW SPECIES OF THE RARE GENUS SCHWARTZITREMA (VIGUERAS, 1940) VIGUERAS, 1941 (TREMATODA: STRIGEIDAE)\*

 $B_{\lambda}$ 

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[Received on 6th January, 1962]

Vigueras (1940) created the genus Schwartziella with Schwartziella schwartzi as the genotype for certain strigeids obtained from the intestine of Anhinga anhinga (Linnaeus). Subsequently, Vigueras (1941) changed the generic name to Schwartzitrem, as the name Schwartzielle was preoccupied for a nematode parasite. Chandler 1951) described the second species viz., Schwartzitrema seamsteri from Fregata magnificens rothschildi, the man-o-war bird. In the present paper the author has described two new species of this rare genus from Indian birds. The genus is being reported for the first time from this country.

#### Schwartzitrema nigericus sp. nov.

About a dozen specimens of this strigeid were obtained from the small intestine of a Little Cormorant, *Phalacrocorax niger* (Vieillot), shot at the outskirts of Hardoi. The infection is quite rare, as a dozen other birds of the same species examined at various places in Uttar Pradesh by the author were found free from these strigeids.

Description: Body (Fig. 1) small and distinctly bisegmented. Forebody bowllike, with a wide terminal opening, 0.513-0.750 mm. in length and 0.432-0.554 mm. in maximum breadth in middle region. Hindbody sac-like, 1.180 mm.-1.694 mm. in length and 0.451-0.712 mm. in maximum breadth in testicular region. Suckers well developed. Oral sucker terminal, 0.078-0.091 mm. by 0.069-0.085 mm. Ventral sucker in posterior half of forebody, 0.091-0.126 mm. by 0.113-0.144 mm. Pseudo-suckers in been, but a pair of slender retractile lobes arise from the inner surface of the dersal wall of forebody and project from the anterior opening of the body (Figs. 1 and 2). The holdfast organ consists of a dorsal main lobe and a pair of ventro-lateral lobes as is characteristic of the genus. The main lobe of holdfast organ deeply netched and confined to posterior half of forebody. Ventro-lateral lobes of holdfast organ long and slender, projecting from anterior opening of forebody. Short prepharynx present. Pharynx muscular, 0.050-0.053 mm. by 0.048-0.053 mm. Short oesophagus present. Intestinal caeca extending upto hind end of body.

Genads in middle of hindbody. Testes large, tandem, transversely elongated. Anterior testis always smaller than posterior one, 0·122-0·190 mm. by 0·261-0·376 mm. Posterior testis 0·160-0·250 mm. by 0·318-0·390 mm. Vas deferens continued into a large saccular seminal vesicle situated just behind posterior testis. Basal part of ductus ejaculatorius dilated to form the so-called "ejaculatory pouch". Ovary pre-testicular, median, transversely oval, 0·100-0·130 mm. by 0·139-)·176 mm. Vitellaria consisting of very dense follicles, extending throughout the length of hindbody and entering posteriorly into the region of copulatory bursa. Follicles mostly aggregated along mid-ventral region of hindbody. Ootype complex inter-testicular. Uterus short with several (eleven to sixteen) eggs.

<sup>\*</sup>Part of the thesis approved for the degree of Doctor of Philosophy at the Lucknow University, Lucknow.

Eggs oval, pale yellow, operculate, 0.0793-0.0887 mm. by 0.0610-0.0700 mm. Uterus joining male duct within the genital cone to form a muscular ductus hermaphroditicus which traverses the cone and opens at its tip. Genital cone large, muscular, protractile, contained within large copulatory bursa which opens to exterior through a terminal pore (Fig. 3).

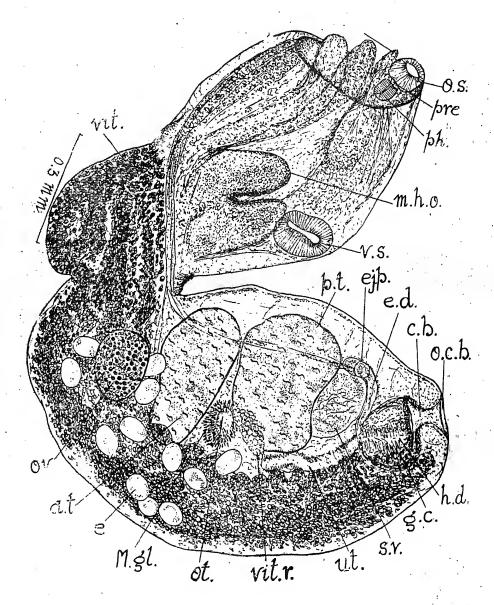


Fig. 1. Schwartzitrema nigericus sp. 1.0v.; type specimen from ventral view

Discussion: The present form closely resembles Schwartzitrema seamsteri Chandler, 1951, but can be distinguished from this species by several features.

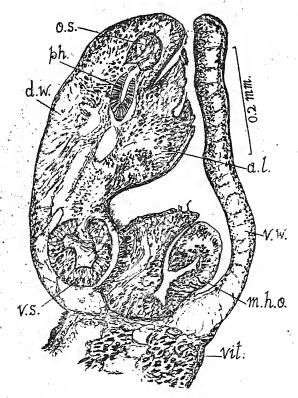


Fig. 2. Schwartzitrema nigericus sp. nov.; sagittal section of forebody.

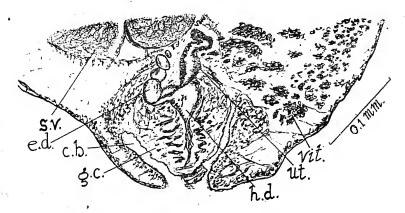


Fig. 3. Schwartzitrema nigericus sp. nov.; sagittal section of posterior end showing copulatory apparatus.

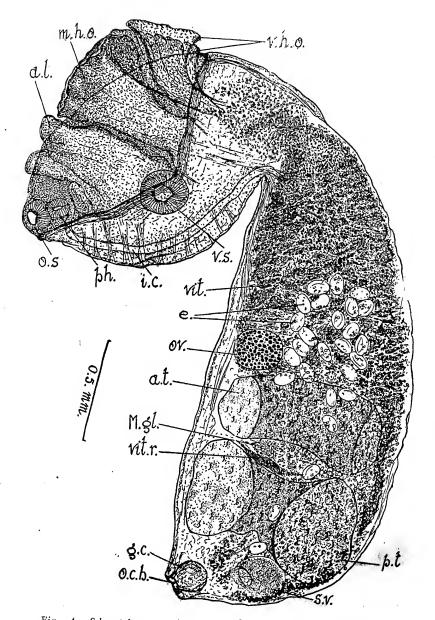


Fig. 4. Schwartzitrema perezi sp. nov.; type specimen from ventral view.

a.l.—anterior protractile lobes; a.t.—anterior testis; c.b.—copulatory bursa; dw.—dorsal wall; e.—eggs; e.d.—ejaculatory duct; ej.p.—ejaculatory pouch; g.c.—gerutal cone; h.d.—ductus hermaphroditicus; i.c.—intestinal caecum; M.gl.—Mehlis' gland; m h o.—main lobe of holdfast organ; o.c.b.—opening of copulatory bursa; o.s.—oral sucker; ot.—ootype; ov.—ovary; ph.—pharynx; pre.-prepharynx; p.t.—posterior testis; s.v.—seminal vesicle; ut.—uterus; v.h.o.—ventro-lateral lobes of holdfast organ; vit.—vitelline follicles; vit,r.—vitelline reservoir; v.s.—ventral sucker; v.w.—ventral wall.

The forebody, in the present form, is bowl-like and not marsupiform as it is in S. seamsteri. A short prepharynx is present in the present form, but it is absent in S. seamsteri. The suckers are considerably smaller in the present form than those of S. seamsteri, although the body size of these worms is approximately equal. The main lobe of the holdfast organ of the present form differs from that of S. seamsteri in being deeply notched anteriorly. Furthermore, the eggs of the present form are considerably smaller than those of S. seamsteri, and the vitelline follicles enter into the region of the copulatory bursa, whereas in S. seamsteri, vitelline follicles do not enter into the zone of the copulatory bursa.

Evidently, the present form represents a new species, and the name Schwartzitrema nigericus is proposed for it.

#### Schwartzitrema perezi sp. nov.

Three specimens of this strigeid were obtained from the small intestine of one out of eight Open-billed Storks, Anastomus oscitans (Boddaert), examined by the author in Lucknow, Hardoi, and Anupshahr during the year 1960.

Description: Body (Fig. 4) large and distinctly bisegmented. Forebody marsupiform with its ventral wall being about one-fourth of dorsal wall in length, and measures 1.374-1.409 mm. in length and 1.239-1.328 mm. in maximum breadth at equatorial region. Hindbody narrow anteriorly, broad posteriorly, with a cone-like caudal end, 2.551-2.577 mm. in length and (.921-1.100 mm. in maximum breadth in testicular region. Suckers well developed. Terminal oral sucker 0.113-0.124 mm. by 0.136-0.142 mm. Ventral sucker in middle region of forebody, 0.158-0.221 mm. by 0.229-0.259 mm. Pseudosuckers absent, but a pair of large protractile lobes arise from the inner surface of the dorsal wall of forebody from the region in front of ventral sucker. Dorsal main lobe of holdfast organ large, pedunculated and funnel-like. Ventro-lateral lobes of holdfast organ long, slender, and highly protractile. Entire holdfast organ conspicuously projecting from the opening of forebody. Prepharynx absent. Pharynx muscular, 0.098-0.116 mm. by 0.087 mm. Oesophagus absent. Intestinal caeca extending upto hind end of body but mostly obscured by vitellaria and other organs.

Gonads in posterior half of hindbody. Testes transversely elongated band like structures folded ventrally at side and thus assuming collar-like shape as in some diplostomes. Anterior testis 0.322-0.370 mm. in length and 0.719-0.800 mm. in breadth excluding the folded parts. Posterior testis always larger than anterior one, 0.520-0.578 mm. in length and 0.849-0.872 mm. in breadth excluding the folded parts. Seminal vesicle subglobular, situated immediatley behind posterior testis. Ovary pre-testicular, lateral, 0.160-0.197 mm. by 0.235-y.249 mm. Vitelline follicles small, extending from basal part of holdfast organ in forebody upto anterior border of copulatory bursa in hindbody. In holdfast organ, follicles present only in ventro-lateral lobes, but not in the main lobe. In hindbody follicles dense in pre-testicular part but sparse elsewhere. Ootype complex intertesticular. Uterus extending a short distance beyond ovary and containing a number of eggs (eighteen to twenty-seven). Eggs oval, yellowish, 0.0910-0.1130 mm. by 0.0547-0.0765 mm. Uterus receiving male duct to form a ductus hermaphroditicus at base of genital cone. Genital cone small. Copulatory bursa small opening out through a narrow terminal pore.

Discussion: The present form shows close resemblance with Schwartzitrema schwartzi (Vigueras, 1940) Vigueras, 1941, but it can be easily distinguished from this species by its oral sucker being larger than the pharynx, by the absence of an oesophagus, by its eggs being smaller than ovary, and by its much smaller

genital cone and copulatory bursa. Moreover, the collar-like shape of the testes in the present torm is so characteristic a feature that it alone distinguishes this form from all other species of the genus Schwartzitrema.

Evidently the present form represents a new species and the name Schwartzitrema perezi is proposed for it, in honour of Perez Vigueras, the author of the genus.

As the new species of the genus Schwartzitrema (Vigueras, 1940) Vigueras, 1941, described in the present paper, show some important features, the diagnosis of the genus is being elaborated as follows:

Emended diagnosis of the genus Schwartzitrema (Vigueras, 1940) Vigueras, 1941.

Strigeidae; Strigeinae; Cotylurini: Body distinctly bisegmented. Forebody cuplike or marsupiform. Hindbody claviform or saccular. Suckers well developed, Ventral sucker larger than oral sucker. Pseudosuckers absent, but instead a pair of protractile lobes, which arise from the pre-acetabular region of the inner surface of the dorsal wall of forebody, is present. The holdfast organ typically consists of a dorsal main lobe and a pair of accessory ventro-lateral lobes. The main lobe is of variable shape. Prepharynx may be present. Pharynx well developed. Oesophagus present. Intestinal caeca extend upto posterior end of body. Testes of variable shape, may be folded ventrally at sides, and placed in middle or posterior region of hindbody. Seminal vesicle saccular. A small ejaculatory pouch may be present at the origin of the ejaculatory duct. Ovary pre-testicular Vitellaria usually confined to hindbody, occasionally entering into the basal part of the forebody. Copulatory bursa with a genital cone and a terminal opening Parasites of intestine of birds.

Type species: Schwartzitrema schwartzi (Vigueras, 1940) Vigueras, 1941.

#### ACKNOWLEDGMENTS

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# EFFECTS OF SOME INSECTICIDES ON THE GUT-EPITHELIA OF CERTAIN INSECTS\*

 $B_{1}$ 

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#### INTRODUCTION

This paper deals with the effects of six insecticides on the gut epithelia of three insects in a comparative and brief manner. Detailed accounts of specific histopathological effects of the particular insecticides on different insects will be published later on separately.

Investigations on the histopathological effects of insecticides on the gutepithelia of insects appear to have been rather neglected. The present study was, therefore, carried out in order to study the effects of some poisons on the gutepithelia of certain insects.

#### MATERIAL AND METHOD

Three insects viz. Leogryllus bimaculatus Sauss., Periplaneta americana Linn. and Gryllodes sigillatus Walk were employed for the present investigations due to their easy availability and omnivorous nature. The insecticides applied were sodium arsenite, lead arsenate, sodium fluosilicate, zinc phosphide, chlordane and benzene hexachloride (B H C). In each case one part of poison was mixed with eight parts of bread and two parts of sugar. Before the insecticidal treatment, the insects were regularly fed on normal food prepared by mixing two parts of sugar to eight parts of bread. These insects were then kept without food for two days to make them sufficiently hungry. Some (control) of these were fed on normal food and dissected at regular intervals after feeding while others were given foods mixed with insecticides and were dissected at the time when they showed symptoms of approaching death. In all experiments the gut pieces were prepared by Yao-Nan and Mann-Kopsch techniques and 5  $\mu$  thick sections were cut. The epithelia from the control insects were examined to see the normal condition of epithelium while those from the treated insects were compared for the study of the changes induced by the insecticides.

# Orservation

The general condition of epithelia in the control specimens of the three insects viz. Leogyllus bimaculatus Sauss., Periplaneta americana Linn: and Gryllodes sigillatus Walk is essentially similar and is represented here by a photomicrograph of a part of midgut epithelium of normally fed specimen of Periplaneta americana Linn. (Fig. 1). The epithelium is seen to be composed of a single layer of columnar cells bounded externally by longitudinal and circular muscles. Just below the younger cells are easily distinguishable groups or nidii of small oval regenerative cells. The cell margins are distinct. The cytoplasm in the cells is finely granular, the granules being finer in the basal regions. The nuclei of the cells generally

<sup>\*</sup>Part of the thesis approved for Ph.D. degree of Lucknow University.

occupy the middle region but their positions may be variable in many cases. The nuclear membrane is always intact and no nuclear extrusion has ever been observed. Generally the columnar epithelial cells retain an even surface at their lumen ends without any trace of attached extruding material over a great length of the epithelium or at times over the entire epithelium. In some cases, however, a few cell extrusions may be observed. These cell extrusions are generally in the form of small or large globular masses of protoplasm. These usually occur in positions occupied by worn-out cells and represent extrusion of these cells.

Insects treated with sodium arsenite, # lead arsenate, # sodium fluosilicate # and zinc phosphide\* (Figs. 2-5) showed more or less complete disintegration of their gut-epithelia as well as the cell cytoplasm. The general effects of the four insecticides in the cases of the three insects remain essentially the same and are represented here by four photomicrographs (Figs. 2-5). The cell margins of the treated epithelia become obliterated. The brush border gets completely destroyed. Lumen ends of the cells break in large numbers allowing extrusion of cytoplasmic globules, irregular masses of cytoplasm, nuclei and even of the entire cell contents; parts of the cells may separate and the portions of the epithelium and even the entire epithelium may separate off from the muscle layers. The nuclei generally remain unaffected by the insecticides but in some cases of poisoning by zinc phosphide\* the chromatin granules appear to arrange along the nuclear membrane of the cells. The effect of chlordane (Fig. 6), though quite severe, remains comparatively milder and the epithelia of the three insects present essentially similar effects as the results of the poisoning by chlordane. In the epithelia from insects poisoned by chlordane (Fig. 6) also, the brush border is completely destroyed as in the case of insects poisoned by the previous four insecticides viz., sodium arsenite, lead arsenate, sodium fluosilicate and zinc phosphide but the disintegration of the cytoplasm in the cells and the obliteration of the cell margins remain comparatively lesser in extent and also the number of the cells with broken lumen ends and the cell extrusions is comparatively small. Benzene hexachloride\* (BHC) has generally no effect on the gut epithelium (Fig. 7) which remains similar to the normally fed individuals in the three insects under investigation but in a few cases production of some cytoplasmic globules has been observed.

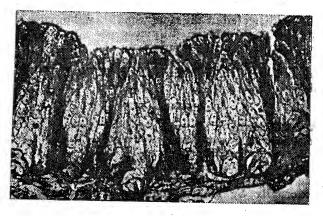


Fig. 1. Photomicrograph of a part from midgut epithelium of normally fed specimen of P. american.

Linn. Mann-Kopsch preparation.

<sup>\*</sup>Concentrations as indicated under 'Material and Method.'

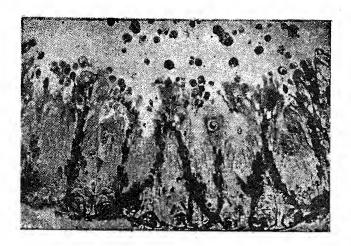


Fig. 2. Photomicrograph of a part from midgut epithelium of *P. amsricana* Linn. poisoned with sodium arsenite. Yao-Nan preparation.

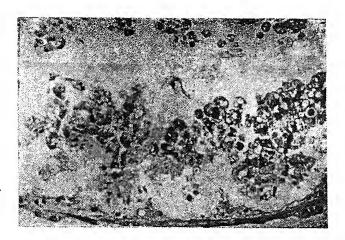


Fig. 3. Photomicrograph of a part from midgut epithelium of *P. americana* Linn. poisoned with lead arsenate. Yao Nan preparation.

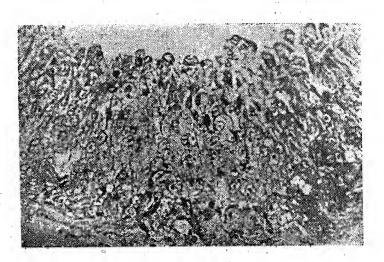


Fig. 4. Photomicrograph of a part from midgut epithelium of G. sigillatus Walk. poisoned with sodium fluosilicate. Yao-Nan preparation.

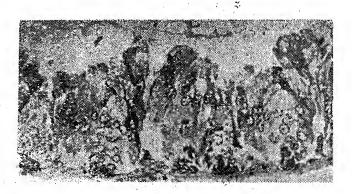


Fig. 5. Photomicrograph of a part from midgut epithelium of *P. americana* Linn. poisoned with zinc phosphide. Yao-Nan preparation.

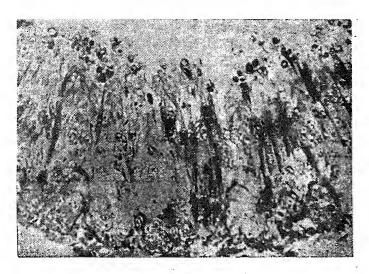


Fig. 6. Photomicrograph of a part from midgut epithelium of G. sigillatus Walk, poisoned with chlordane. Yao-Nan preparation.

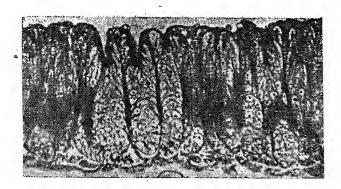


Fig. 7. Photomicrograph of a part from midgut epithelium of G. sigillatus Walk. poisoned with B H C. Mann-Kopsch preparation.

#### DISCUSSION

Results of some earlier workers on the effects of these poisons on the gutepithelia of certain other insects were as follows. Pilat (1935) described the destruction of epithelium in midgut of Vanessa urticae and Locusta migratoria following the ingestion of sodium arsenite and sodium fluosilicate. Wilson (1936) observed destruction of midgut epithelium in the larva of Pieris rapae following the ingestion of sodium arsenite. Woke (1940) showed disintegration of epithelium in midgut of Prodinia eridania following the ingestion of lead arsenate. Day and Powning (1949) described marked epithelial break-down by arsenic compounds in Blatella. Day and Powning (1949) also found that chlordane produced loss of striated border, separation of epithelial cells and cytolysis and that benzene hexachloride caused formation of vacuoles in the cells. Formation of vacuoles was also observed by Srivastava (1948) in the nerve cells of Blatella.

#### **ACKNOWLEDGMENTS**

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## COMPARATIVE STUDIES IN POLLEN GRAIN GERMINATION OF CERTAIN PLANTS OF MALVACEAE AND RELATED FAMILIES IN VITRO

By

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#### INTRODUCTION

The germination of pollen grains of Malvaceae has been studied by Amici (1830), Guignard (1904), Stenar (1925), Lang (1937), Iyengar (1938), Purewall and Randhawa (1947), Verma and Verma (1957) and Datta (1958) and that of Tiliaceae has been investigated in detail by Datta (1957). In the present day in vitro germination studies of pollen grains, application of different substances like verious sugars, aminoacids and vitamins has been introduced and their effects in different concentrations may be interpreted for the economic exploitation of increased seed production by enhancing the percentage of germination of the pollen grains and by accelerating the rate of development of the pollen tubes and subsequently by increasing the final length of these tubes. The pollen grains of Malvaceae are polyforate while those of Tiliaceae and Bombacaceae are planaperturate. Datta (1958) has reported polysiphonous pollen tubes in certain species of Malvaceae. He observed as many as eleven tubes emerging out of a single pollen grain in case of Hibiscus vitifolius L. and twenty-six tubes in the case of Hibiscus esculentus L. Iyengar (1938) also noted polysiphonous germination of the pollen grains in Hibiscus vitifolius L. and Purewall and Randhawa (1947) observed polysiphonous and branched pollen tubes in Hiliscus esculentus L. Under the present study the percentage of germination of the pollen grains of the species under investigation has been acerta ined and the rate of development of the pollen tubes has been found out. Besides, a critical study of the type of germination in vitro has been made.

#### MAIERIALS AND METHODS

Pollen grains of Hibiscus vitifolius L., Hibiscus esculentus L., Hibiscus mutabilis L., Hibiscus rosa sinensis L., Sida spinosa L., Abutilon indicum G.Don., Malachra capitata L., Althaea rosea L., Bombax malabaricum DC. and Grewia asiatica L. were collected soon after anthesis and dusted on slides smeared with sucrose-agar-gelatin medium (1 gm sucrose, 5 gm agar, 25 c.c. water and 5 gm gelatin). The slides were placed into the humid chambers of petridishes. So humidity during the time of germination of the pollen grains was maintained at hundred per cent. The laboratory temperature varied between 26 and 27°C. The growing pollen tubes were measured at very frequent intervals and finally at the end of an hour after dusting. The germinated and ungerminated pollen grains were counted and percentage of fertility of the pollen grains was calculated out for each species from a random counting of three hundred pollen grains. The fertility of the pollen grains was directly acertained by staining them with acetocarmine. The stained grains are taken as the fertile ones while the unstained and the shrivelled pollen grains are considered

to be sterne. For this purpose tive hundred pollen grains of each species were examined.

## OBSERVATION

In all the cases where germination of the pollen grains took place in vitro, it started almost within ten minutes of dusting and the day hours were found to be more suitable than the morning or evening hours for germination.

The pollen tubes of Hibiscus vitifo ius L. are polysiphonous. No branching of the tubes was observed. However, rare branching of the tubes has been reported by Datta (1958). The largest number of pollen tubes emerging out of a single pollen grain was found to be twelve. The growth of the pollen tubes was very rapid between the tenth and the thirtieth minutes after dusting. After thirty minutes the growth slowed down and no further growth took place after one hundred minutes. The pollen tube measuring  $108.50~\mu$  at the end of thirty minutes measured only  $121.52~\mu$  and  $125.86~\mu$  at the ends of sixty and one hundred minutes respectively. The following lengths of different individual tubes were noted at the end of an hour:  $43.40~\mu$ ,  $65.40~\mu$ ,  $86.80~\mu$ ,  $121.52~\mu$  and  $173.60~\mu$ . The longest tube recorded was  $173.60~\mu$ . 95% of the pollen grains germinated in the artificial medium while 97.65% pollen grains were found to be fertile when stained with acetocarmine.

The germination of pollen grains of Hibiscus esculentus L. was also found to be of polysiphonous type and the tubes were profusely branched. The largest number of tubes developed from a single pollen grain was twentyone. The growth of the pollen tubes was very rapid during the first half an hour after dusting of the pollen grains. The growth of the tubes practically ceased after one hour. The pollen tube measuring 120540  $\mu$  at the end of thirty minutes measured 1483·10  $\mu$  at the end of an hour. Individual tubes measured 1205·60  $\mu$ , 1407·30  $\mu$ , 1483·10  $\mu$ , 4560·90  $\mu$ , and 1557·00  $\mu$  at the end of an hour. The last one was the longest individual tube observed. 94% of the pollen grains germinated in vitro. 96% of them were found to be fertile when tested with acetocarmine.

The pollen tubes of *Hibiscus mutabilis* L. are also polysiphonous. No branching of the tubes was observed. The largest number of tubes emerging out of a single grain was found to be seven. The growth of the pollen tubes was very rapid during the first half an hour of dusting of the pollen grains. There was no growth after an hour of dusting. The pollen tube measuring  $60.30~\mu$  at the end of thirty minutes measured  $75.78~\mu$  at the end of an hour. The following lengths of individual tubes were noted at the end of an hour:  $43.40~\mu$ ,  $47.74~\mu$ ,  $73.78~\mu$ , the pollen grains germinated in vitro, but 87.5%, when stained with acctocarmine, were found to be fertile.

In the case of Sida spinosa L. the pollen tubes were found to be monosiphonous and unbranched as a rule, but a few pollen grains also produced two tubes. The growth of the pollen tubes was relatively faster during the first thirty minutes of dusting. Thereafter it gradually retarded and finally it ceased after an hour. The pollen tube measuring  $27.30~\mu$  at the end of half an hour measured  $34.62~\mu$  at the end of one full hour. At the end of an hour the individual tubes measured:  $16.40~\mu$ ,  $17.36~\mu$ ,  $17.48~\mu$ ,  $34.62~\mu$  and  $43.40~\mu$ , the last one being the longest tubes found. Germination was only 40% in vitro, but cent per cent pollen grains were found to be fertile on being stained with acetocarmine.

Only 4% of the pollen grains of Abutilon indicum G.Don. germinated in vitro, while hundred per cent were found to be fertile when tested with acetocarmine. The pollen tubes were monosiphonous and unbranched. The growth was faster during the first half an hour of dusting of the pollen grains. After an hour there was practically no further growth of the pollen tubes. The pollen tube measuring  $30.40~\mu$  at the end of thirty minutes measured only  $38.50~\mu$  at the end of sixty minutes. Individual pollen tubes measured  $12.50~\mu$ ,  $19.70~\mu$ ,  $36.40~\mu$ ,  $38.50~\mu$  and  $41.30~\mu$  at the end of an honr. The last one was the longest tube recorded.

The pollen grains of Hibiscus rosa sinensis L., Malachra capitata L., Althaea rosea L., Bombax malabaricum DC. and Grewia asiatica L failed to germinate either in the medium or in 01% boric acid or in sucrose solutions of different concentrations up to 7%. But 68.60%, 100%, 100%, 98.40% and 66.83% pollen grains respectively of the above species were found to be fertile when tested with acetocarmine. Datta (1958) has also reported the failure of germination of the pollen grains of Althaea rosea L. in vitro, but he has reported germination of the pollen grains of Malachra capitata L.

The result of the observations are tabulated below:

	Percentage of germination	Type of germination	Longest tube observed
Hibiscus vitifolius L. 97-65	95	Polysiphonous, no branching, up to 12 tubes observed emerging out of a single pollen grain.	173·60 µ
Hibiscus esculentus L. 96	94	Polysiphonous, tubes profusely branched.	1557•00 μ
Hibiscus mutabilis L. 87-5	20	Polysiphonous, no branching.	86·80 µ
Sida spinosa L. 100	40	Monosiphonous, no branching.	43·40 µ
Abutilon indicum G.Don. 100  Hibiscus rosa-sinensis 123 68:60	4 No germination	Monosiphonous, no branching.	41·30 μ
Malachra capitata L. 190	3)		
Althaea rosea L. 100	"		
Bombax malabaricum DC. 98·40 Grewia asiatica L. 66·83	» .		

#### **SUMMARY**

- 1. The pollen tubes of *Hibiscus vitifolius* L., *Hibiscus esculentus* L. and *Hibiscus mutabilis* L. are polysiphonous and those of *Hibiscus esculentus* L. also exhibit branching.
- 2. Pollen grains of Sida spinosa L. and Abutilon indicum L. are monosiphonous and unbranched. In case of the former one very sparingly the pollen grains may germinate by means of two pollen tubes.
- 3. Pollen grains of Hibiscus rosa-sinensis L., Malachra capitata L., Althaea rosea L., Bombax malabaricum DC. and Grewia asiatica L. did not germinate in vitro.
- 4. In all cases where in vitro germination of the pollen took place the growth of the pollen tube was rapid during the first half an hour of dusting of the pollen grains. This was followed by a slow period of growth and finally the cessation after an hour's time. Such observation has also been made by Datta (1957) in the species of Corchorus, wherein he reports that the growth of the pollen tubes was rapid during the first one hour after germination.
- 5. Day hours suited best for in vitro germination of the pollen grains in all the cases.

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# PRELIMINARY STUDIES ON THE HUMUS STATUS OF SOME FOREST COMMUNITIES OF BASHAHR HIMALAYAS

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#### INTRODUCTION

Humus is highly important from the stand point of forest growth and production, controlling to a great extent the physical, chemical and biological properties of the soil. The significance of humus in a forest soil can be determined not so much by its abundance as by the rapidity of its decomposition (Lutz and Chandler, 1959). In a normal developing forest the decomposition of the organic debris must keep pace with the addition of fresh material from the forest vegetation (Waksman, 1938). It is also opined (see Waksman, 1938) that humus layer conditions tend to be different under different forest types. Thus a type of humus layer of a soil which formerly supported hard woods may change when a pure crop of conifers is established on the land. In light of these observations the present studies were made to evaluate the humus status under different forest communities in the Bashahr Himalayas during the month of June, 1960.

#### HUMUS

There is some controversy regarding the application of the term 'humus'. Waksman (1938) has defined humus as a 'complex aggregate of brown to black coloured amorphous substances, which have originated during the decomposition of plant and animal residues'. Lutz and Chandler (1959) regarded humus as "the plant and animal residues of the soil; litter excluded which are undergoing evident decomposition". However, certain German investigators (Albert, 1929) differentiated three successive products in the formation of forest humus, viz.: (a) litter, (b) surface humus or raw humus, and (c) soil humus. The 'F' (Formultingsskiktet) and 'H' (Humusämneskiktet) layers (Hesselman, 1925; Lutz and Chandler, 1959) of the raw humus are not well differentiated in the Indian forests. Therefore, in the present communication the author prefers to identify the organic debris above the soil surface excluding the fresh litter as 'unincorporated humus' and the humus present in the soil as 'soil humus'.

#### AREA OF STUDY AND METHODS

The Bashahr Himalayas include the Lower Bashahr and the Upper Bashahr Forest Divisions of Himachal Pradesh and are bounded by Tehri Garhwal in the south and Seraj Division of Punjab in the north. The area is mountaneus with rugged topography, and experiences typical Himalayan climate. Present studies were mostly confined to the Lower Bashahr Himalayas, the physiography, climate and biota of which have already been discussed in detail (Mohan and Puri, 1954).

At selected places in the area forest communities were formed by noting down the frequency of each associate in 5 metre circular quadrats marked along a transect running from the foot hill to the top. In each community 10 quadrats of a square metre dimension were marked at random. Fresh litter from each

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quadrat was collected, separated into twigs, leaves and cones and weighed directly in the field. Similarly unincorporated humus was collected and weighed from each quadrat. Soil samples were also collected and their humus content was evaluated in the laboratory by the method adopted by De Sigmond as described by Navalkar (1959).

#### OBSERVATIONS AND DISCUSSION

Data is summarized in the table 1. The maximum amount of fresh litter is observed under Abies-Picea Taxus-Quercus community and the lowest under Pinus wallichiana community. Leaf litter forms the major portion of the forest litter, though the amount of twig-litter is also considerable especially under Abies-Picea-Taxus Quercus community. Highest amount of cone-litter is seen under Picea smithiana community and the lowest under the young plantation of Cedrus deodara. This may show the high reproductive capacity of the Picea smithiana stand. The quantity of cone-litter is also quite low under the Abies-Picea Taxus-Quercus community which otherwise shows maximum total litter.

There is a tendency of accumulation of the unincorporated humus at higher altitudes under similar vegetation types. This is especially exhibited by the Pinus wallichiana community which was examined at two different altitudes. At Narkanda (2780 m.) the amount of unincorporated humus under this community is much more (1003.09 g. per sq. metre) than that near Bai Bahli (2420 m., 466.552 g. per sq. metre). There is not much difference in the amount of unincorporated humus under the two conifer communities, viz., Picea smithiana and Cedrus deodara, growing at approximately similar height. The presence of the trees of Abies tindrow and Taxus baccata in the forest communities seems to favour the accumulation of the unincorporated humus. This is indicated in the Abies Picea-Taxus Quercus and Abies-Picea-Pinus communities. The Cedrus-Pinus community, situated at a little lower altitude than the Pinus wallichiana (II) community, also shows more unincorporated humus than the latter. The amount of unincorporated humus is comparatively quite low under the Quercus semecarpifolia community.

The ratio of total fresh litter/unincorporated humus and the amount of soil humus (in the surface soil) are highest under Querous semecarpifolia community. This may show the higher speed of litter decomposition and rapid incorporation of the decomposed material into the soil for further mineralization under this community than under pure confers. As it is often observed that the leaves decompose rather rapidly than twigs, etc., the ratio of leaf, twig amongst the fresh litter assumes some importance in determining the rate of decomposition. But in the present study this ratio does not give a clear indication except in the case of Quercus semecarpifolia community where it assumes the highest value.

The presence of broad-leaved species like Quercus semecarpifolia and Rhododen-dron arboreum and the abundance of shrubs of Indigofera gerardiana and Rosa macrophylla in the conifer communities seem to favour the decomposition of coniferous litter. This is indicated by high values of the ratio of total fresh litter/unincorporated humus and the percentage of soil humus under Abies-Picea-Taxus-Quercus and Pinus wallichiana (II) communities. The importance of low shrubs, capable of living happily under the canopy of the main tree species and furnishing a litter attractive to the soil animals is well recognised for the maintenance of a balanced forest (Anderson, 1956). Litter under somewhat young plantation of Cedrus deodara with high leaf/twig ratio also seems to be favourable for rapid decomposition. This is possible partly, because the litter of young trees

TABLE 1

Humus status of some forest communities of Bashahr Himalayas

# (Grams per sq. metre)

		(1			1			S TOTAL	0 :	Section 1	
		in) (əi		Fresh litter		giw	Total	Unincor-	Total/	, h	ST
Comminity	Locality	BuilifA	Leaves	Twigs	· Cones	Leaf/T	litter	humus	unincor- porated humus	surface soft	e Remarks
Pinus wallichiana (I)	Narkanda	2780	326.587	221-612	139-966	14.1	688 165	1003.09	£0.68	2.96	With scattered trees
Abies-Picea-Pinus	Narkanda	2780	612:352	332.419	209.948	1.83	4154-719	2093-65	.0.22	5.26	
Abies-Picea-Taxus- Quercus	Baghi	2690	1078-902	6294850	151.49	11.2.1.	1772-902	2052-83	98.0	08 9,	21 tl.
Picea 'smithiana	Khadrala	2870	629-850	277.444	250~770	2.76	1108-064	1580.44	£0.73	.6.20	With shribstof Row macrophylla.
Cedrus deodra	Khadrala	7870	863-120	349.914	52.487	2.46	1265-521	1446.31	0.87	6.43	Young! plantation,
Pinus wallichiana (LI)	Bai Bahli	2420	367.410	204-117	151.629	1.80	723-156	466*55	1.55	7.15	With scattered trees of Rhododendron
											undergrowth of Indigofera gerardiana
Cedrus-Pinus	Nankhari	2360	513-210	180.789	93-310	2.83	787.309	.991-42	. 08 • 5	2.00	
Quercus semecarpifolia	Baghi	2710	921-440	250-772	1	3.67	1172-212	589.05	1.99	10.78	( * * * * * * * * * * * * * * * * * * *
			*								

is generally more tender and palatable to the soil organisms (Anderson, 1956), which help in its rapid break down.

Indicated below, are some of the factors possibly responsible for the slowness in the decomposition of litter causing, thereby, accumulation of the unincorporated humus under coniferous communities.

Close Canopy: Coniferous communities usually have close canopy, due to which light does not ordinarily reach the surface of the soil. This may also decrease the temperature at the soil surface and cause slowness in the microbial activity which is so very intimately connected with the process of decomposition. This close canopy is especially noticed in the forest communities having trees of Abies and Taxus. Pure communities of Picea smithiana and Pinus wallichiana have comparatively open canopy.

Structure of the litter layer: The layer of the fresh litter under Quercus semecarpifolia community has a loose structure with large interstitial spaces, because the broad leaves of the oak trees are often curled or contorted when dry. On the other hand, litter layer under pure conifer community, formed by closely lying needles, is compact with less of interstitial spaces. The presence of broad-leaved trees and shrubs in the conifer communities imparts somewhat intermediate structure. The importance of loose litter with greater amount of interstitial spaces has been recognised by many European workers including Heatwole (1961) These large interstitial spaces permit free activity of animals on the forest floor and increase the aeration, essential for the activity of aerobic bacteria. Moreover, these allow greater penetration of light and rain water rendering the conditions more favourable for rapid decomposition of the litter and its incorporation into the soil.

Chemical composition of fresh litter: The nature and the chemical composition of the fresh material reaching the soil surface are also important, determining, to a great extent, the rate of litter decomposition (Lutz and Chandler, 1959). The lower C/N ratio and higher calcium content in the leaves of broadleaved species than the conifers (Puri and Gupta, 1951) make the conditions more favourable for rapid decomposition of broad-leaved litter. The litter with high contents of basic mineral salts is also preferred by the various organisms which feed upon the forest litter (Anderson, 1956) helping in its decomposition.

#### SUMMARY

Humus is highly important from the stand point of forest growth and production. With a view to investigate quantitatively the humus status under different forest communities of Bashahr Himalayas, the studies reported and discussed in the present paper were made during the month of June, 1960. The author prefers to identify the organic debris above soil surface excluding fresh litter as 'unincorporated humus' and the humus present in soil as 'soil humus'.

The ratio of total fresh litter/unincorporated humus and soil humus (in surface soil) are highest under Quercus semecarpifolia community. This may suggest that the rate of decomposition of forest litter is higher under oak (Quercus semecarpifolia) community than under coniferous communities. The presence of broadleaved species like Quercus semecarpifolia and Rhododendron arboreum and the abundance of shrubs of Indigofera gerardiana and Rosa macrophylla in the conifer communities seem to fovour the decomposition of coniferous litter. The probable reasons for the slowness in the decomposition of coniferous litter are discussed.

#### ACKNOWLEDGEMENT

The author wishes to express his grateful thanks to the Chief Botanist, Botanical Survey of India and Dr. G. S. Puri, the then Director, Central Botanical Laboratory, Allahabad for the necessary facilities and encouragement.

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<sup>\*</sup>Not seen in original.

# ON NEW FAMILY LAMPRITREMATIDAE OF SUPERFAMILY HEMIUROIDEA FAUST, 1929, WITH A KEY TO THE FAMILIES OF THE SUPERFAMILY

By

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[Received on 28th November, 1961]

The subfamily Lampritrematinae Yamaguti, 1940 created for the genus Lampritrema Yamaguti, 1940 does not belong to the family Hemiuridae Luhe, 1901. Its very long thick muscular body covered with thick cuticle, bulbous oesophagus, caeca provided with stomach portion at the beginning and saccular outgrowths in posterior region, tubular convoluted vesicula seminalis covered in its distal region with thick muscular coat, and cirrus sac enclosing strongly convoluted ductus ejaculatorius opening along with metraterm in large genital atrium ventral to male papilla, and long tubular excretory vesicle with strongly winding lateral collecting ducts recurrent at both ends of body are such characters as completely exclude it from the Hemiuridae. A new family Lampritrematidae is, therefore, created for its reception. This family stands among such specialised families as Hirudinellidae Dollfus, 1932, Sclerodistomidae Dollfus, 1932 and Prosogonotrematidae Pérez Vigueras, 1940 containing large distomes with thick muscular body covered with thick cuticle and characterised by several features of genital ducts.

## Lampritrematid ae n. fam.

Family diagnosis.—Hemiuroidea Faust: Body very long, narrow muscular with thick cuticle, papillated in anterior part. Suckers well developed. Acetabulum large very prominent, pre-equatorial, some distance behind intestinal bifurcation. Oral sucker surmounted by preoral lobe. Prepharyn absent; oesophagus bulbous; caeca lined with cuticle and forming bulb like stomach portion at commencement, and developing sac like outgrowths posteriorly, terminating blindly at hinder end. Genital pore ventral to pharynx. Genital atrium large muscular. Testes tandem at about midbody. Seminal vesicle tubular convoluted in front of acetabulum. Cirrus sac present enclosing strongly convoluted ductus ejaculatorius. Ovary post-testicular, median. Receptaculum seminis present. Laurer's canal absent. Vitellaria two, long narrow branched winding tubes extending backward along outer side of caeca to about half way between ovary and posterior extremity. Uterus extending behind vitellaria. Metraterm muscular opening into genital atrium ventral to male papilla. Ductus hermaphroditicus absent. Eggs numerous, embryonated. Excretory vesicle long tubular. Collecting excretory ducts lateral, strongly winding, recurrent at both ends, not united anteriorly. Parasitic in stomach of marine fishes.

Type genus: Lampritrema Yamaguti, 1940

Genotype and single species L. nipponicum Yamaguti, 1940

The family stands apart from Hemiuridae on account of the long muscular body covered with thick cuticle, presence of bulbous stomach at commencement of

queca and saccular outgrowths near posterior end, presence of cirrus sac enclosing long strongly convoluted ductus ejaculatorius and absence of ductus hermaphroditicus. The metraterm is muscular and opens separately in large genital atrium ventral to male papilla. Excretory vesicle tubular and long; excretory collecting ducts lateral, strongly winding, extending anteriorly and posteriorly from the excretory vesicle. This family is closely related to families Hirudinellidae Dollfus, 1932 and Sclerodistomidae Dollfus, 1932 which are of large stout distomes with thick cuticle and possess stomach part at commencement of caeca. The vesicula seminalis is tubular and winding. The pars prostatica is tubular and winding in the latter two families as well as in Lampritrematidae n. fam. The ovary is post-testicular in these families. The receptaculum seminis is present in the new family, but it is absent in Hirudinellidae and Sclerodistomidae, in which Laurer's canal is present, the latter absent in Lampritrematidae n. fam. In Hirudinella oral sucker is surmounted by fleshy lip, in Lampritrema the oral sucker is surmounted by preoral lobe. The posterior saccular outgrowths of caeca of the latter may represent in an incipient condition the huge posterior inflated part of caeca of Hirudinella ventricosa Baird, 1853 described by Manter (1947).

Manter (1947) says "Dollfus proposed that the Sclerodistominae be raised to family rank and separated from Hirudinella largely on the basis of excretory system. Considering the terminal genital ducts, the tubular vitellaria the shell gland complex and the similarity in body form I am inclined to retain Sclerodistomum and Hirudinella in the Sclerodistominae. In both genera the excretory, vesicle is v.luminous either as much coiling tubes or as bulbous inflated tube". Nigrelli and Stunkard (1947) also do not agree with Dollfus in the erection of the families for the genera Hirudinella and Bathycotyle etc. They prefer to retain the subfamilies Hirudinellinae and Bathycotylinae for them until their life histories are known. We agree with Dollfus (1933) that the families of Hemiuroidea, Syncoeliidae Odhner, 1928, Accacoeliidae Looss, 1912 and Bathycotylidae Dollfus, 1932 are valid. We also believe that in the present state of knowledge there is a case for maintaining the families Hirudinellidae Dollfus and Sclerodistomidae Dollfus. The life histories are not expected to be known soon and to wait for their knowledge to keep these taxonomic questions in abeyance does not appear to be a proper method.

It is clear that the two families Hirudinellidae and Sclerodistomidae are very closely related. A stomach part is present at the commencent of caeca in both of them and Lampritrematidae n. fam. The ovary is post-testicular. Receptaculum seminis is absent in Hirudinellidae and Sclerodistomidae, but present in Lampritrematidae, in which Laurer's canal is absent. Vitellaria are branched or tubular; divided into several long slender winding tubules in Sclerodistomum. The excretory vesicle is long and tubular, and common lateral excretory ducts are winding recurrent at both ends in the Lampritrematidae n. fam. The excretory system of the latter family is somewhat primitive, from which the excretory systems of the Hirudinellidae Uollfus, 1932 and Sclerodistomidae Dollfus, 1932 may have been evolved. Manter's point of view, seems to be justified to a certain extent as the latter two families are closely related to one another and to Lampritrematidae n. fam. which is primitive and apparently connects them.

According to the diagnosis of Hirudinellidae Dollfus given by Yamaguti, the strongly muscular cirrus pouch of *Hirudinella* (Garsin, 1730) contains ejaculatory duct and metraterm, and the genital pore is ventral or posterior to pharynx; whereas in Sclerodistomidae the genital pore lies midventral between two suckers and the genital cone encloses hermaphroditic duct and opens into large genital

atrium. In view of the fact that the metraterm opens separately from the male papilla in genital atrium in Lampritrema, it shows a primitive condition and probably stands intermediate between that of the ancestor of Sclrodistomidae and of the latter family itself in which the hermaphroditic canal lies enclosed in the genital cone. I am inclined to believe that the modification of the male papilla of Lampritrema into the genital cone of Sclerodistomum enclosing hermaphroditic duct is a later condition evolved from that in Lampri!rema. The so called cirrus sac of Hirudinellidae should be called muscular sac as it contains both ductus ejacula torious and metraterm. The cirrus sac of Lampritrematidae n. fam. is thus differentiated from it. Dollfus's paper (1932) in which he has raised the Sclerodistomatinae to the family Sclerodistomidae, separating it from the Hirudinellidae is not available to me and my information is from the diagnosis of these families given by Yamaguti (1958). It appears that families Sclerodistomidae Dollfus and Hirudinellidae Dollfus, though closely related, are tenable. In the description of Sclerodistomum sphaeroides Manter, 1947 the statement "the metraterm leads through the compact stroma surrounding the seminal vesicle and joins the male duct in the genital cone" shows the condition is intermediate between that in Lampritrema and that met with in the Sclerodistomidae.

The genus Prosogonotrema Pérez Vigueras, 1940 (Family Prosogonotrematidae Pérez Vigueras, 1940) having robust cylindrical body with thick cuticle, tubular winding vesicula seminalis and convoluted ductus ejaculatorious, the latter imbeded in muscular tissue also comes near Lampritrema in that the terminal parts of its male and female ducts run in its large genital cone contained in the genital atrium. This condition stands parallel to that of Lampritrema in which the metraterm opens ventrally to the male papilla. Prosogonotrema also possesses posttesticular ovary and very long slender tubular vitellaria running mainly ventral to caeca. Its excretory system is reported to be Y-shaped with arms united anteriorly. Prosogonotrena, Lampritrema, Hirudinella and Sclerodistomum are a series of specialised genera showing close affinities to one another. Lampritrema in this series occupies a more or less central position between Prosogonotrema on one side and Hirudinella and Sclerodistomum on the other. Prosogonotrema differs from them all in lacking a stomach part of the caeca and in its Y-shaped excretory system. The genus Lampritrema Yamaguti resembles Hirudinella (Garsin, 1730) more closely than Sclerodistomum Looss, 1912 in great length of body and position of genital pore ventral to pharynx. But it differs from it in the presence of cirrus sac in contrast to the muscular sac of Hirudinella, in its vitellaria and excretory system.

Nigrelli and Stunkard (1947) find that in *Hirudinella* the relation of the terminal parts of both male and female ducts enclosed in a common muscular sac are subject to great variations due to its retractions and extensions. The male and female ducts may open into a long and deep genital sinus due to retraction or they may open on a common genital protrusion of the muscular sac.

In Hirudinella spinosa Yamaguti, 1938, the two caeca become gradually expanded behind the posterior end of the uterus and become ultimately united with each other as well as the excretory vesicle in the hind body. The collecting excretory vessels extend winding their way inside the subcuticular layer in complicated coils in hind body and then enter the intercaecal field to wind their way in forebody where they are united with one another on the dorsal surface of the oral sucker in a manner similar to that slown by Poirier (1835) in Distomum clavatum. The disposition and structure of the terminal genital ducts also agree with those described in the latter species. It is thus clear that Hirudinellidae, Sclerodistomidae, Prosogonotrematidae and Lampritrematidae n. fam. differ in their excretory system.

Ley to families of superfamily Hemiuroidea Faust
Cirrus sac present
1. Cirrus sac containing vesicula seminalis and pars prostatica; excretory vesicle Y-shaped with long S-shaped stem
Cirrus sac containing very long convoluted ductus ejaculatorious only; convoluted long vesicula seminalis free; excretory vesicle long tubular with long common collecting ducts recurrent at ends:  Lampritrematidae n. fam.
2. Vitellaria branched; excretory vesicle Y-shaped with long stem and very long cornua; parasitic in marine snakes
Genital sinus large, muscular complicated with folds and separate male and female pores on protrusible papilla at its bottom; vitellaria follicular; excretory vesicle small with two main canals forming U-shaped system; parasitic in Selachii
3 "Museuler and present"
3. Muscular sac present 4 Muscular sac absent 5
4. Muscular sac enclosing ductus ejaculatorious and metraterm; genital pore ventral or posterior to pharynx; caeca distended posteriorly excretory vesicle with strongly winding lateral collecting ducts
5. Genital pore between suckers; hermaphroditic duct passing through genital cone in genital atrium; caeca not distended posteriorly; excretory vesicle V-shaped
Terminal parts of male and female ducts passing through large genital cone in genital atrium; stomach absent; excretory vesicle Y-shaped with arms united anteriorlyProsogonotrematidae Perez Vigueras 1940.
Testes divided into follicles or irregularly segmented tubules
Testes two not follicular
6. Ovary slender tubular; stomach part present
Ovary not tubular; stomach absent

<sup>\*\*</sup>Oesophagicolidae n. fam. connects Hemiuroidea Faust, 1929 with Opisthorchioidea Faust, 1929.

<sup>\*\*\*</sup>Ptycogonimidae Dollfus and Arnolidae n. fam. stand intermediate between Hemiuro'dea Faust and Azygioidea Skrj. and Guschanskaja, 1956.

7. Intestinal caeca H-shaped with anterior outgrowths reaching pharynx and posterior caeca opening into excretory vesicle..... ......Accacoeliidae Looss, 1912. Intestinal caeca ordinary, not H-shaped......8 8. Cuticle thick; body robust; acetabulum encircled by fold of body wall; ovary intertesticular......Bathycotylidae Dollfus, 1932. Cuticle not thick; body not robust; acetabulum ordinary; not encircled by fold; ovary post-testicular or pre-testicular..... REFERENCES Dawes, B. 1956. The Trematoda. Cambridge Univ. Press: 1-644. Dollfus, R.Ph. 1933. Sur quelques Parasites de poissons recoltes a Casti-glione (Algerie.) Bull. Trav. pub. Station d' Agricult. et Peche de Castiglione, Ann. 1933, fas. 2: 200-224. 1937. Trematodes de selachians et de cheloniens. Bull. Comite d'etudes Hist. scient. de l'Afrique Occident. Française. Paris 19 (4): 397-519. 1937. Les Trematodes Digenea des Selaciens (Plagiostomes) Catalogue par Hotes. Distribution Geographique. Ann. Parasit. Hum. Comp. Faust, E. C. 1949. Human Helminthology. Henry Kimpton, London. 1-744. Fuhrmann, O. 1928. Trematoda. In Kukenthal und Krumbach. Handbuch d. Zool. 2. Part 2: 1-140. La Rue, G. R. 1957. Parasitological Reviews. The classification of digenetic trematoda: A review and new system. Experim. Parasit. 6 (3): 306-349. Lühe, M. 1909. Parasitische Plattwurmer: I. Trematoden. susswasser fauna Deutschl. Heft 17: 1-215. Manter, H. W. 1947. The digenetic trematodes of marine fishes of Tortugas. Florida. Amer Midl. Natur. 38 (2): 257-416. Nigrelli R. F. and Stunkard, H. W. 1947. Studies on the genus Hirudinella Giant trematodes of scombriform fishes. Zoologica, Soc. 31 (4): 185-196. Yamaguti, S. 1938. Studies on the helminth fauna of Japan. Part 21. Trematodes of fishes, IV. Published by author. Kyoto Japan: 1-140. 1958. Systema Helminthum. Vol. I. The digenetic trematodes of Vertebrates. Parts I and II. Interscience Publishers, Inc., New York. · . . 1-1575.

# ADDITIONS TO THE FUNGI OF JABALPUR (MADHYA PRADESH)-II

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In the first series of the paper the author has described eight Fungi imperfecti occuring at Jabalpur. Some more fungi are being described here which include two new species, two new hosts, and some new records from the state.

The serial numbers correspond to the additional fungus flora of Jabalpur.

9. Colletotrichum pancratiae Hasija sp. nov. on leaves of Pancratium sp. College garden, August 1961, Leg. Hasija.

#### Symptoms of the disease:

The disease starts as small ash coloured irregular spots which later increase in size. The spots are surrounded by light brown margin. Black pinehead like accervuli appear in the central infected regions. The spots are midrib limited and their coalescence is rare.

#### The causal organism:

Acervuli broad, light brown, superficial,  $48.5-198 \,\mu$  wide, with scattered setae on the acervulus; setae dark brown, septate; conidia hyaline, single celled, cylindrical to oval,  $3.3-5.6 \, \times 1.7-2.5 \,\mu$ . (Fig. 1)



1. Colletotrichum pancratiae-Acervulus with conidia.

The specimen was examined by Mr. Sutton, Assistant Mycologist, Commonwealth Mycological Institute, Kew. So far no Colletotrichum has been described on any Pancratium. The present fungus is being represented here as a new species Colletotrichum Pancratiae

#### Colletotrichum pancratiae Hasija sp. nov.

Acervuli lati, pallide brunnei, superficiales,  $48.5-198 \,\mu$ ; setis dispersis per acervulum, brunneis, septatis; conidia hyalina, unicellularia, cylindrica vel ovalia  $3.3-5.6\times1.7-2.5 \,\mu$ .

<sup>\*</sup>Now re-named as Government Science College.

In foliis viventibus Pancratiae sp. ad Jabalpur in India, mense Aug. 1961, Leg. Hasija.

The type specimen has been deposited in the Herbarium of the Commonwealth Mycological Institute, Kew. No. 88881.

10. Colletotrichum graminicola (Ges) Wilson, Phytopath., 106, 1914.

On leaves of Urochloa reptans Beauv., Water works, Sept. 1960, Leg. Hasija.

# Symptoms of the disease:

The disease first appears as small, dark brown, scattered spots. At advanced stage the spots become irregular and change to ash colour. The acervuli become perceptible as black dots in this region. Rarely these spots may coalesce.

## The causal organism:

Acervuli brown, superficial, 66-148 µ wide; setae brown, septate; conidia hyaline, sickle shaped, single celled, 9·9-25·4×3·3-4·8 μ.

Colletotrichum graminicola is a very common pathogen and is found on the leaves of Andropogon sorghum throughout India. It has been recorded from Jabalpur by Agarwal, Nema and Beliram (1959). Urochloa raptans is a new host record for the fungus from India.

The specimen has been deposited in C. M. I. Kew, Herbarium No. 83927.

11. Pestalotiopsis japonica (Syd.) Stey. in Bull. Jard. Bot. Brux., 19: pp. 285-354, 1949. On leaves of Terminalia sp., Katau, August, 1961, Leg. Hasija. 

# Symptoms of the disease:

Disease appears as yellow decolouration of leaf, which later changes to light brown. The infection is restricted on the upper surface only. Spots are irregular in which black dot like acervuli appear at maturity. Often the lesions may coalesce. Midrib acts as barrier.

# The causal organism:

Acervuli brown, broad 90-165  $\mu$  wide; conidia ellipsoid to fusoid, 4 septate, end cells hyaline, central cell dark coloured, with three cilia at apical end, 14·1- $22 \times 4.9 - 8.2 \mu$ , average  $20 \times 6.5 \mu$ .

Pestalotiopsis japonica has been earlier reported on the leaves of Ficus glomereta from Jabalpur by Agarwal and Hasija (1961). So far there is no record of the fungus on any species of *Terminalia*. The present *Terminalia* sp. is a new host record for the fungus from India.

The species was identified by Mr. Sutton, Assistant Mycologist, C. M. I. Kew. The specimen, has been deposited in the I. M. I. Herbarium No. 89441 (a). 12. Helminthosparium capense Thumen, in Flora, lix, p. 570, 1876. On leaves of Citrus limonia, Waterworks, November, 1960, Leg. Hasija.

The fungus was growing as hyperparasite on a species of Meliola on the surface of Citrus leaves.

Conidiophores dark brown, septate, simple or branched, with geniculations, in fascicles, bearing conidia successively on new growing tips,  $66-297 \times 5 \cdot 6-6 \cdot 6 \mu$ , (average 188 × 6 μ); conidia light coloured, usually 2-5 septate, more or less obclavate, truncate at the base which bears a scar, smooth,  $23 \cdot 1 - 49 \cdot 5 \times 6 \cdot 6 - 9 \cdot 8 \mu$ , (average  $38.5 \times 9 \mu$ ).

This hyperparasite has been reported to be growing on the colonies of various Meliolines on a wide range of hosts. It is the commonest hyperparasite in tropical Africa. Helminthosporium capense has been reported as parasitic on colonies of Meliola salaciae Hansf. from Bangalore by Thirumalachar and Lacy (1951). This is a new record for the state.

The specimen has been deposited in the I. M. I. Herbarium No. 84366.

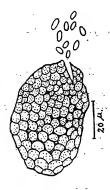
13. Phyllosticta microconidiai Hasija sp. nov. on leaves of Pogostemon plectranthoides Desf. Berhaghat, April, 1961, Leg. Hasija.

#### Symptoms of the disease:

Disease initially appears as light brown pinhead like scattered spots. Spots are irregular with ash coloured central region and a dark brown halo. Coalescence of spots is rare. They are restricted on either side of the midrib.

#### The causal organism:

Pycnidia dark brown, superficial, globose to subglobose,  $39-132 \mu$  in diameter, (average  $85.5 \mu$ ); conidia hyaline, single celled, oval,  $3.3-6.5 \times 1-1.8 \mu$ , (average  $4.8 \times 1.2 \mu$ ). (Fig. 2).



2. Phyllosticta microconidiai-Pycnidia with conidia.

Phyllosticta pogostemonis Cochr. (1951) has been described on leaves of Pogostemon sp. from U. S. S. R. The present species differs from P. pogostemonis in having smaller pycnidia and conidia, therefore the present fungus is being described here as a new species Phyllosticta microconidiai.

## Phyllosticta microconidiai Hasija sp. nov.

Pycnidia brunnea colore, superficiales, globosa vel subglobosa, diametientia  $39-132~\mu$ , mediet  $85.5~\mu$ ; conidia hyalina. semel cellulata, ovalia,  $3.3-6.5\times1-1.8~\mu$ , mediet  $4.8\times1.2~\mu$ .

In foliis viventibus Pogostemonis plectranthoidesis ad Jabalpur in India, mense April, 1961, Leg. Hasija.

The material was examined by Mr. Sutton, of C.M. I. Kew and the type specimen has been depsited in I. M. I. Herbarium No. 86173.

14. Parodiella hedysari (Schw.) Hughes in Mycol. pap. C. M. I. 48, 1952.

On leaves of Desmodium triflorum D. C., College ground, Sept. 1960, Leg. Hasija. The perithecia are seen scattered on the upper surface of the leaf. Perithecia dark brown, superficial, globose, smooth, appendages not present, 96-298 \mu in diameter, average, 185 \( \mu \); asci hyaline several, club shaped, with rounded apex, with 8 ascospores in each ascus, 60-125 \mu in length, average 93 \mu; ascospores light brown. two celled, elliptical, 15-25.6  $\times$  6.4-9.6  $\mu$ , average 21  $\times$  7  $\mu$ .

Desmodium is one of the favourite hosts of Parodiella hedysari. Parodiella perisporioides (Burk and Curt) Speg. which is a synonym of P. hedysari (Schw) Hughes, is reported from all parts of the country, on this host. This is a new record for the state.

The species was identified by Mr. Pirozynski of C. M. I. Kew. The specimen has been deposited in the I. M. I. Herbarium No. 84687.

#### SUMMARY

The present paper describes six fungi occuring at Jabalpur. It includes. Colletotrichum pancratiae Hasija sp. nov. on leaves of Pancratium sp. and Phyllosticta microconidiai Hasija sp. nov. on leaves of Pogostemon plectranthoides the two new species. Urochloa reptans for Colletotricium graminicola (Ces) Wilson and Terminalia sp. for Pestalotiopsis japonica (Syd) Stey. are the two new hosts record from India. Helminthosporium capense Thumen, a hyperparasite on Meliola sp. on the surface of the Citrus leaves and Parodiella hedysari (Schw) Hughes on Desmodium triflorum are new records for the state.

#### **ACKNOWLEDGEMENTS**

The author expresses his grateful thanks to Dr. G. P. Agarwal, Botany Department, Government Science College, Jabalpur, for his encouragement and guidance, to Dr. J. C. F. Hopkins, Director, Mr. Sutton, Mr. Pirozynski, Assistant Mycologists, Commonwealth Mycological Institute, Kew, England for the identification of the species, to Rev. Fr. Prof. H. Santapau, Chief Botanist, Botanical Survey of India, Calcutta, for kindly rendering into latin the diagnoses of the new species. Thanks are also due to Prof. U. D. Mukherjee, Principal, and to Prof. S. K. Verma, Head of the Botany Department, Government Science College, Jabalpur, for the laboratory facilities.

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# TWO NEW SPECIES OF THE GENUS TYLODELPHYS DIESING, 1850 (TREMATODA: DIPLOSTOMATIDAE) FROM INDIAN BIRDS\*.

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[Received on 6th January, 1962]

Tylodelphys Diesing, 1850 is a small diplostome genus, including, at present, only five species. In the present paper the author has described two more species from Indian birds. The genus is being reported from India for the first time.

#### Tylodelphys spinnata sp. nov.

Five specimens of this diplostome were obtained from the small intestine of an Open-billed Stork, *Anastomus oscitans* (Boddaert), shot at the outskirts of Lucknow.

Description: Body (Fig. 1) bisegmented; segments not well marked. Forebody linguiform, 0.940-1.120 mm. long. Hindbody bluntly conical, 0.845-0.890 mm. long. Maximum breadth of body 0.550-0.746 mm. at junction of body segments. Suckers moderately developed. Oral sucker 0.072-0.091 mm. by 0.090-0.110 mm. Ventral sucker situated at about middle of forebody, 0.081 mm. by 0.073-0.091 mm. Pseudosuckers well developed, 0.075-0.106 mm. by 0.067-0.081 mm. Holdfast organ, with a median narrow slit-like opening, situated in hind region of forebody, 0.310-0.431 mm by 0.368-0.391 mm. Adhesive gland masked by vitelline follicles. Pharynx longitudinally oval, 0.068-0.079 mm. by 0.059-0.067 mm. Short prepharynx and oesophagus present.

Testes large, contiguous, occupying greater part of hindbody; broader than long and folded ventrally at sides, appearing collar-like. Anterior testis 0·182-0·267 mm by 0·431-0·568 mm, and posterior one 0·191-0·296 mm, by 0·370-0·542 mm., excluding the folded parts. Seminal vesicle coiled, situated immediately behind posterior testis. Ovary pretesticular, situated laterally in region where two segments of body are confluent; globular or subglobular, 0·139-0·207 mm, by 0·139-0·196 mm. Vitelline follicles small, extending from a level midway between pharynx and ventral sucker upto the region of seminal vescile, never entering the region of copulatory bursa. Ootype complex inter-testicular. Uterus with few (usually two) eggs. Eggs large, light yellow, 0·0962-0·0970 mm, by 0·0674-0·0751 mm. Uterus receives male duct to form ductus hermaphroditicus which passes through a well developed genital cone and opens at its tip. Genital cone located in a small copulatory bursa, which communicates with the exterior through a wide, subterminal aperture.

Discussion: The present form closely resembles Tylodelphys americana (Dubois, 1936) Dubois, 1937 than the other species of this genus. It can, however, be easily distinguished from T. americana by its ventral sucker being larger than its pharynx. Moreover, the ovary in the present form is globular or subglobular, and eggs are only few in number, whereas in T. americana the ovary is ellipsoidal, and eggs are numerous. Further, the present form has more pronounced pseudosuckers, smaller and poorly demarcated copulatory bursa, and larger genital cone. Thus, the present form represents a new species and is named as Tylodelphys spinnata.

<sup>\*</sup>Part of a thesis approved for the Ph.D. degree of the Lucknow University, Lucknow.

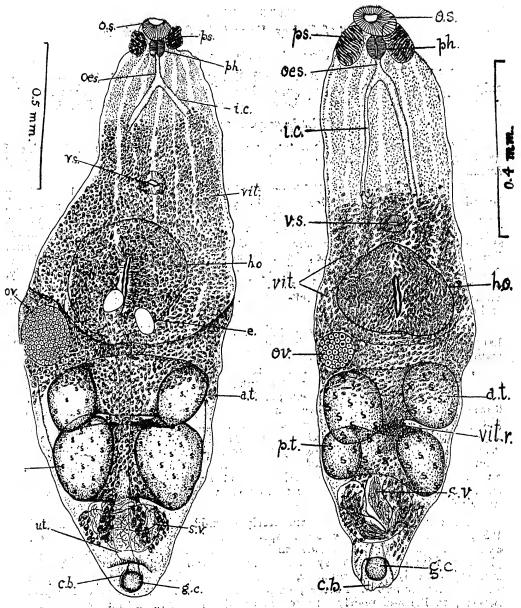


Fig. 1. Tyladelphys spinnala sp. nov ; type specimen from ventral view.

Fig. 2. Tylodelphys indica sp. nov.; type specimen from ventral view.

at.—anterior testis; c.b.—copulatory bursa; e.—eggs; g.c.—genital cone; h.o.—holdfast organ; i.e.—intestinal caecum; oes.—oesophagus; o.s.—oral sucker; ov.—ovary; ph.—pharynx; ps.—pseudosuckers; p.t.—posterio testis; s.v.—seminal vesicle; ut.—uterus; vit.—vitelline follicles; vit.r.—vitelline reservoir; v.s.—ventral sucker.

#### Tylodelphys indica sp. no.

About three dozen specimens of this diplostome were obtained from the small intestine of the Indian Darter (The Snake Bird), Anhinga melanogaster Pennant. The infection seems to be quite common, as six birds out of nine examined at Anupshahr, Hardoi, and Lucknow, were found infected with this fluke.

Description: Body (Fig. 2) bisegmented; segments not well defined. Forebody linguiform, with lateral margins slightly folded ventrally in posterior region, 0.769-1.106 mm. long. Hindbody bluntly conical, 0.471-0.565 mm, long. Maximum breadth of body 0.327-0.410 mm., in region of holdfast organ. Oral sucker large, 0.069-0.081 mm. by 0.080-0.110 mm. Ventral sucker delicate, situated just behind middle of forebody, 0.040-0.051 mm. by 0.059-0.062 mm. Pseudosuckers prominent, oval, 0.098-0.120 mm. by 0.054-0.077 mm. Holdfast organ with a narrow slit-like opening placed medially in posterior region of forebody, 0.194-0.211 mm. by 0.231-0.272 mm. Adhesive gland obscured by vitelline follicles. Short prepharynx present (seen only in well extended specimens). Pharynx longitudinally oval, 0.051-0.060 mm. by 0.051 mm. Short oesophagus present. Intestinal caeca extending upto posterior end of body.

Testes large, situated in middle of hindbody, broader than long with sides rolled ventrally and thus appearing collar-like. Anterior testis always longer than posterior one, 0·108-0·122 mm, by 0·261-0·332 mm, excluding folded parts. Posterior testis 0·085-0·097 mm, by 0·231-0·280 mm, excluding folded parts. Seminal vesicle coiled, placed just behind posterior testis. Ovary immediately pre-testicular, usually dextral, spherical or subspherical, 0·081-0·084 mm, by 0·089-0·091 mm. Vitelline follicles small, extending from a short distance in front of ventral sucker upto the region of copulatory bursa. Ootype complex inter-testicular. Eggs (Fig. 3) few, present in some specimens only, 0·716-0·0826 mm, by 0·0512-0·0527 mm. Uterus receives male duct to form a ductus hermaphroditicus which traverses a prominent genital cone. Genital cone situated in a large copulatory bursa opening out through a wide terminal pore.

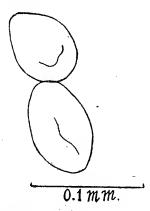


Fig. 3. Eggs of Tylodelphys indica sp. nov.; from a paratype specimen.

Discussion: Of all the species of the genus Tylodelphys Diesing, 1850, the present form most closely resembles T. excavata (Rudolphi, 1803) Szidat, 1935, from which it can be chiefly distinguished by the fact that its anterior testis is longer than the posterior one, whereas in T. excavata, the posterior testis is longer than the

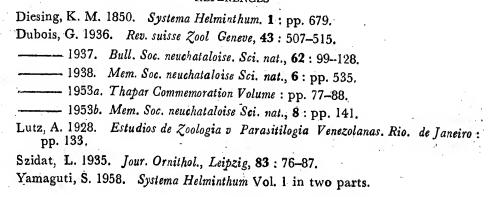
anterior one, and also by the larger size of its pseudosuckers. The present form can be further distinguished from T. excavata by its vitellaria which do not enter into the anterior half of the forebody, whereas in T. excavata, the vitellaria enter into the anterior half of the forebody for a considerable distance.

Evidently, the present form represents a new species, and the name Tylodelphys indica is proposed for it.

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# A COMPARISON OF SOIL FUNGAL FLORA) OF THREE DIFFERENT GRASSLANDS

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#### INTRODUCTION

Several workers have shown that the soil fungal flora differs in different environments. The work relates to the study of soil fungi from (i) cultivated soils (Jaseveli, 1924; Jensen, 1931; Chaudhury and Sachar, 1934; Chand, 1937; Singh, 1937a; Ghatak and Roy, 1939), (ii) from forest soils (Pain, 1927; Pistor, 1930; Ellis, 1940; Saksena, 1955), (iii) from soils with high salanity (Bayliss-Elliot, 1930; Sabet, 1935; Killian and Feher, 1935), (iv) from chalk and lime bed soils (Nicholes, 1956; Shetye, 1956), (v) from cultivated comparing with the natural soils (Dixon, 1928; Janke and Holzer, 1929; Bisby et al, 1933; Ma; 1933a), and (vi) from different depths in a particular soil (Pain, 1927; Swift, 1929; Todd, 1932; Bisby et al, 1935; Deyl, 1938; Warcup, 1951). The fact that fungi differ from season to season in a particular soil has been discovered by Dixon (1928), Ma (1933) and Svinbufvud (1937).

Variation in the fungal flora from different vegetational areas has been found by several modern workers. Tresner et al (1954) have concluded that there is a series of progressively changing species combinations along the gradient of the upland hardwood forest continuum of Southern Wisconsin. The same result has been obtained by Orpurt and Curtis (1957) who worked on ecological relationship between surface vegetation and micro-organisms of Wisconsin. England and Rice (1957) compared the soil fungi of a tall grass prairie and of an abandoned field and concluded that these habitats harboured different fungal flora.

#### MATERIAL AND METHODS

In the present investigation the study of soil fungal flora of three grass plots has been undertaken. The plots, supporting different dominant grass species, viz. (A) Desmostachya bipinnata Stapf.—Cynodon dactylon Pers. association, (B) Vetiveria zizanioides Nash.—Dichanthium annulatum Stapf. association and (C) Saccharum spontaneum Linn. with some annual weeds respectively, are situated in the same field with similar set of conditions about thirty yards apart from one another. This study has been undertaken with a view to investigate whether there exists any qualitative or quantitative change in the soil fungal flora of the grass plots.

Fungi were isolated at the depths of 0-6", 6-12" and 12-18" in the month of May, 1959 and methods of soil sampling, isolation and purification of fungi were same as mentioned by Dwivedi (1958).

#### RECORD OF FUNGAL FLORA

TABLE I
(Distribution of fungi at various depths)

## Grass plot (A)

NT a C a		Depths						
Name of species	0-6"	6–12"	12-18"					
Rhizopus nigricans	+	+ '	+					
Mucor luteus	+	_	_					
M. circinelloides	. +	- ·						
M. ohlongisporus	#	+	•					
M. saturninus	+							
Syncephalastrum racemosum	+	_	· ·					
*Eremascopsis spinosa gen. et sp. nov.	-	+	-					
Neocosmospora vasinfecta	· · +	+						
Aspergillus nidulans	_ ·	+ , .	291					
A. niveus	म	++	-					
A. terreus	+	+	+					
A. niger	* -1-	+	+					
A. awamori	. +	.+-	***					
Penicillium javanicum	+	+	+					
P. funiculosum	+	+	-					
Trichoderma viride	+	+						
Cladosporium herbarum	+	+	476					
Stysanus medius	+	+	+					
Gurvularia lunata	+	+	_					
Alternaria humicola	+	+						
Humicola fusco-atra	+	+,						
Epicoccum duriaenum	+	•	-					
Golletotrichum falcatum .,	+	_ *						
Pestalotia sp.	* +	+						

NOTE 1—Authorities for all binomials of fungi are as given by Gilman (1945). Thom and Raper (1945), Raper and Thom (1949) and Saccardo (1884). Authorities for such species which are not given in these books have been mentioned in footnote.

<sup>2-\*</sup>Proc. Indian Sci. Congr. 1960, p. 320.

<sup>3-+=</sup>species present, -=species absent.

TABLE II
(Distribution of fungi at various depths)

# Grass plot (B)

Name of species			Depths	
		0-6"	6–12′′	12-18"
Rhizopus nigricans		+	+	-
Mucor luteus		+	-	· .
Thielavia terricola		+	+	_
Neocosmospora vasinfecta		+	+	-
Aspergillus nidulans		+	+	_
A. variecolor	• •	+	+	8.
A. terreus		+	+	-
A. candidus		+	+	+
A. niger		+	+	+
A. flavus		_ +	+	<del>-</del>
A. sydowi		•	+	÷ <del>-</del> .
A. japonicus		.+ .	-	
A. sulphureus		+	*	<del>-</del> '
Aspergillus sp.		+	e * •	+
Penicillium funiculosum			+	-
P. humicola		+	•••	+
Penicillium sp.	• •	+	<b></b>	<del></del> '
Gephalosporium coremioides		+	+	-
Paecilomyces variotae		+	+	4
*P. fusisporus		+	<del>-</del>	
Gladosporium herbarum		+	+	<b>.</b>
Alternaria humicola	1.	+	+	
Helmint'iosporium anomalum		+	+	
Curvularia lunata		+	+	
Fusarium nivale		+	+	+

<sup>\*</sup>P. fusisporus Saksena.

TABLE III
(Distribution of fungi at various depths)

## Grass plot (C)

Name of species	Depths				
name of species	Pullinganian dist. A		0-6''	6-12"	12–18"
Rhizopus nigricans	F		+	·+	and a second
Mucor sp.			+		_ * :
**Royella albida gen. et sp. n	ov.		÷		100
Neocosmospora vasinfecta			+	+	
Aspergillus nidulans			+	<b>.</b>	<b>.</b>
A. variecolor			·	:	
A. niveus			<u>.</u>	-L	
A. candidus			<b>'</b> 45	l 	
A. terreus	4,			.1	. —
A. niger			T L	<del>-</del>	- The state of the
A. sulphureus					
†Penicillium raistrickii				-	<del>-1-</del>
P. funiculosum		,		710	, see
†P. spiculisporum	<u>.                                    </u>				* Heral 9 5 7 9 90
Penicillium sp.	2.5			T.	-
Trichoderma viride	• • •		+	+	-L
*†Hendersonula toruloidea			-	+	T
†Paecilomyces fusisporus			+	- <u>-</u>	<del></del>
Gephalosporium sp.	***		+	4	· *
Stachybotrys sp.			<u>.</u>		
*†Phialophora richardsiae				<u>.</u>	
Cladosporium herbarum	Ă.		+	-T	T ·
Helminthosporium halodes			-L-	- <del>-</del> .	
*Spegazzinia tessarthra			+	<u> </u>	
Scolecobasidium constrictum			·	-	- <del></del>
*† Myrothecium verrucario	• •		<u>.</u>	 I:	T 14,774
†Fusarium chlamydosporum			丁	+	
Mycelia sterilia	• •		· <del>-  </del> -	- 1	1
	****		5	+	

<sup>\*</sup>Authorities for\* are Nattrass, (Nauff.) Conant, (Berk. and Curt.) Sacc. and Ditmar ex Fr

<sup>\*\*</sup>Proc. Indian Sci. Congr. 1960, p. 320-21.

<sup>†</sup>Deposited in Commonwealth Mycological Institute, Kew, England.

TABLE IV
(Physico-chemical characters of soil)

Glass plots	Depths (inches)	pН	Moisture content	W.H.C. %	Organic matter%	Available nitrogen%	NO <sub>3</sub> Mg/ 100 gm. soil	Exch. Ca m.e. %	Exch. Na m.e. %	No. of fungi per gm. of dry soil
	0-6	7·1	8.95	52.6	0 9	0.008	12	5.34	0.26	70,000.
A	6-12	6.8	9.74	55.3	3.2	1:022	10.6	5.64	0.3	76 <b>,</b> 666
	12-18	7.1	10.94	53.6	0.19	. 0.019	8.5	4.98	0.66	26,666
	0-6	7.1	7.2	43.5	0.93	0.001	9	6.7	1:68	30,000
В	6-12	7.1	7.5	44.3	1.2	0.012	9.6	6.7	0.48	23,809
	12-18	7.1	8.7	47.6	0.21	0.001	9.7	7-4	0.68	8,231
	, 0–6	7.2	<b>7</b> ⊨ .	44.6	- 2.9	0 019	8.4	8.6	0.6	19,717
. <b>G</b> .	6-12	7.1	8.6	40.9	2.4	G•12 ·	8.2	6.84	1.16	9,700
	12-18	7.1	8 6	37.1	1.7	0.19	7:3	7.84	0.96	9,203

W. H. C.—water holding capacity.

TABLE V

Number of genera and total number of species occurring in different soils

Grass plot	Phycor	nycetes		Ascomycetes Genera Species		omycetes	Total No
	Genera	Species	Genera	Species	Genera	Species	of species
Α	3	6	4.	4	. 11	14	24
. B	. 2	2	4	4	9	19	. 25
C	2	2	4	. 5	15	21	28

#### DISCUSSION AND CONCLUSION

From the data given in tables I to IV it will be clear that all types of vegetational sites harbour particular types of fungal populations and different number of fungi per gram of soil. The number of fungi per gram of soil in the plot (A) is greater at all depths than that of other plots (Table IV). Fungi isolated from this plot are three genera and six species from the Phycomycetes, four genera from the Ascomycetes (including ascigerous Aspergillus and Penicilium) and eleven genera and fourteen species from the Deuteromycetes (Table V). From the plot (B) only two genera and the same number of species from the Phycomycetes, four ascogenous genera, nine genera and nineteen species from the Deuteromycetes have been discovered. The largest number of Aspergilli have been obtained from this plot. In case of the plot (C) the number of fungi per gram of soil is the least at all depths but it excels in variety of species. The number of genera from the Mucorales and from the Ascomycetes are two and five respectively, while the Deuteromycetes are represented by fifteen genera and twenty-one species. The Deuteromycetes are remarkable in the sense that not only they are dominant but rather represent the variation in and the largest number of varieties. Thus all the plots support the growth of fungi differing in kind and number per gram of dry soil and only a few viz., Rhizopus nigricans, Neocosmospora vasinfecta, Aspergillus nidulans, A. terreus and A. niger are common to all of them. Moreover, two new ascogenous genera, viz., Eremascopsis spinosa gen. et sp. nov. and Royella albida gen. et sp nov. have been discovered from the plots (A) and (C) respectively.

Fungi are abundant in the first horizon and decrease according to the increase in depth. It is in accordance with the observations of Goddard (1913), Takahasi (1919), Cobb (1932) and Warcup (1951). Exception in case of the plot (A) is noteworthy in the fact that in the middle horizon number of fungi per gram of soil is greater than the upper one (Table IV), which may be considered due to higher percentage of water holding capacity, organic matter and available nitrogen. The least number of fungi per gram of soil in the plot (C) further support the observation made with regard to the plot (B) that the same factors are responsible for this decrease.

In this investigation the change in the surface vegetation in different plots is accompanied by the change in the variety of fungi. This is in conformity with the observations of Tresner et al (1954), Thornton (1956), Orpurt and Curtis (1957) and England and Rice (1957). Martin and Ervin (1958) concluded that there was some change in fungal flora when old Citrus soils were cropped to orange seedlings. The authors' observation shows that the variety of soil fungi may be influenced by root excretions of surface vegetation and this effect varies in proportion to the variety of vegetation present. The conclution arrived at by authors and other workers in the field and considerable weight to the concept of distinct fungal flora of different vegetational areas.

#### SUMMARY

A comparative study has been made on summer soil fungal flora of three grass plots supporting different dominant grass species; viz., (A) Desmostachya bipinnata Stapf.—Cynodon dactylon Pers. association, (B) Vetiveria zizamoides Nash.—Dichanthium annulatum Stapf. association, and (C) stands of Saccharum spontaneum Linn. respectively. It has been discovered that the change in vegetational sites is accompanied by similar change in the soil fungal populations both quantitatively and qualitatively and only a few forms like Rhizopus nigricans, Neocosmospora vasinfecta, Aspergillus nidulans, A. terreus and A. niger have been found to be common to all the plots. Physico-chemical characters of soils have also been estimated.

We are grateful to Prof. R. Misra, F. N. I. for his kind encouragement and for providing all facilities.

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# HISTOPATHOLOGICAL CHANGES IN THE LIVER OF CERTAIN FISHES AS INDUCED BY BHC AND LINDANE

By

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[Recieved on 23rd April, 1962]

Although Laug et al. (1950) have described that histopathological changes could be detected in the liver of rats at a very low concentration of DDT, however, Haag (1948) and Greenwood (1953) failed to find such pathological changes. Woodard and Hagen (1947) reported liver damages following the feeding of gamma isomeres of BHC to dogs.

Histopathological study of tissues damaged or of change occurring in fishes exposed to BHC and Lindane, is of great economic importance. The histopathological changes in the liver, as a result of these insecticides, have been studied in the present work. The liver was fixed and sectioned for the preparation of microscopic slides and stained with haematoxylin and eosin. No pathological changes similar to those of experimental liver were noted in the control slides. The pathological changes caused by BHC and Lindane on the liver of different fishes are described below:

#### 1. BHC.

# (a) Ophicephalus punctatus:

BHC produced malignant liver damages. The chief alteration consisted of hypertrophy in the central area of the liver. Necrosis could also be detected. Near the periphery a few parenchymatous cells had disappeared completely. Here and there a few spaces had been created due to the degeneration of these cells. The cells were deformed and the whole organ looked like an aggregation of cells (Fig. 2).

#### (b) Heteropneustes fossilis:

The microscopic changes in the liver were of two types.

- (i) hepatic cells alterations.
- (ii) alterations that were less specific but often of more damaging nature, such as diffused hepatic cells distribution, hypertrophy, atrophy and necrosis of hepatic cells. The lesions were more pronounced in the central than in peripheral area.

Due to the degeneration of hepatic cells a few spaces appeared in the centre of the cells. Degeneration of hepatic cells was more marked. There was too much clumping of a few liver cells and the whole area looked like a complicated mass of cells. The polygonal shape of the cells was deformed. The nucleus was also swollen due to the effect of BHC and slightly displaced from its original central position. There was a tendency of peripheral cells to stain more deeply than the central ones. Hypertrophy of cells was more pronounced (Fig. 3).



Fig. 1. Photomicrograph of T.S. of Normal fish, liver cells, showing the usual pattern of cell size, and cytoplasmic granules (con rol slide). X100.



Fig. 2. Photomicrograph of T. S. of infected liver from BHC (Ophicephalus punctatus), showing hypertrophy and atrophy. (X100).



Fig. 3. Photomicrograph of T. S. of infected liver from BHC of Heteropneustes fossilis showing necrosis and hypertrophy (X450).



Fig. 4. Photomicrograph of T. S. of infected liver from BHC of Trichogaster fasciatus (X450).



Fig. 5. Photomicrograph of T. S. of infected liver from BHC of Barbus stigma (X100)

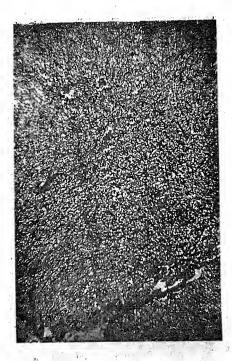


Fig 6. Photomicrograph of T. S. of infected liver from Lindane of Ophicephalus punctatus (X100).

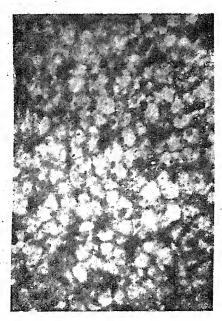


Fig. 8. Photomicrograph of T. S. of infected liver from Lindane of Trichogaster fasciatus (X450).

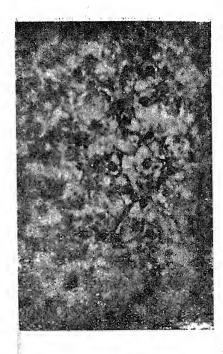


Fig. 7. Photomicrograph of T.S. of infected liver from Lindane of Heteropneustes fossilis (X450).

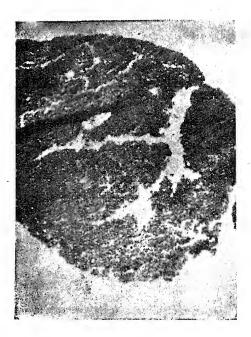


Fig. 9. Photomicrograph of T. S. of infected liver from Lindane of Barbus stigme (X100).

# (c) Trichogaster fasciatus :

The liver cells showed marked degeneration. The hypertrophy was not so much marked. The cytoplasm of the cells disintegrated and due to its absence the cells became hollow and looked like vacuoles. There was localised necrosis in a few hepatic cells. At places the peripheral hepatic cells were more dense and took deeper stain. The polygonal charater of the hepatic cells was deformed. There was much alteration in hepatic cells (Fig. 4). The liver cells became vacuolated and the cytoplasm gave the appearance of a network. There was marked atrophy.

# (d) Barbus stigma:

The hepatic cells were slightly vacuolated and the vacuoles being very small. There was marked hypertrophy. At few places, localised necrosis of hepatic cells was found. There was a tendency in a few cells of clumping together. Near the periphery a few cells had disappeared completely (Fig. 5).

#### 2. Lindane.

#### (a) Ophiocephalus punctatus:

The hepatic cells were moderately vacuolated the vacuoles being small or large. In a small area there was a slight atrophy of cells in the peripheral and central region. The central hepatic cells stained less deeply than the peripheral ones. The pathological change was in shape of liver damage. There was localised necrosis of the hepatic cells leading to hypertrophy. The centre of the cell appeared empty due to degeneration of the cytoplasm of the cells. The peripheral cells were more dense (Fig. 6).

# (b) Heteropneustes fossilis:

The affected liver cells were scattered in distribution. At higher dosages (50 p. p. m.) margination was characteristic. Margination of cells was characteristically associated with the cells hypertrophy. There was necrosis of hepatic cells. The hepatic cells were moderately vacuolated. The centre of the cells appeared empty due to the loss of cytoplasm.

The change in parenchymatous cells of the liver consisted of margination of cytoplasmic granules. The nucleus was slightly displaced from its original central position. At places necrosis was very much pronounced. At few places the cells had degenerated completely. There was also a tendency in the hepatic cells to clump together (Fig. 7).

# (c) Trichogaster fasciatus:

The pathological changes were the same as in the previous case. The hapatic cells were markedly vacuolated, the vacuoles being small and large. Due to degeneration of cytoplasm, the centre of the cells become hollow. At places there was also degeneration of hepatic cell. The lesions were more marked in the central area. The polygonal shape of the cells was deformed. The peripheral cells were more dense and there was much accumulation of stain in that area (Fig. 8).

#### (d) Barbus stigma:

The hepatic cells showed marked degeneration and at places the cells had disappeared completely. Necrosis was well pronounced. The chief alteration was the hypertrophy of hepatic cells (Fig. 9).

#### SUMMARY

- 1. The chlorinated hydrocarbon insecticides, BHC and Lindane were chosen for the study of histopathological changes in the liver of certain fishes.
- 2. Lesions could be detected in the liver at very low dosages of BHC (10 p.p.m.) and Lindane (5 p.p.m.) but at higher dosages (50 p.p.m.) these were more pronounced.
- 3. The changes in the parenchymatous cells of the liver consisted of margination, hypertrophy and the vacuolation of the hepatic cells.
- 4. The chief alterations induced by BHC were the hypertrophy, necrosis and atrophy.
- 5. By the inducement of Lindane the hepatic cells exhibited vacuolation, necrosis and hypertrophy.

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# FUNGI CAUSING PLANT DISEASES AT MUZAFFARPUR-1

 $B_{\gamma}$ 

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The following is a list of fungal diseases so far collected at Muzaffarpur (Bihar State) since January, 1960. The fungi reported below have been collected from living parts of the hosts. Regular monthly surveys of various localities in this town have been undertaken at short intervals. The additions to this list of pathogenic fungal flora will be published in subsequent supplements. The herbarium specimens are being deposited in the Botany Department of the L. S. Gollege, Muzaffarpur.

#### PHYCOMYCETES

- 1. Albugo bliti (Biv.) de Bary (Reported as Cystopus bliti in Fungi of India-Butler and Bisby, 1931) on leaves of Amarantus viridis L., Leg. S. S. Pd., Mithanpura, 10-8-61.
- 2. Albugo candida (Pers.) Lev. (Sacc. VII: 234) on leaves of Brassica campestris L., Leg. B. D. Sinha, Khabra Road, 5-3-6.
- 3. Peronospora arborescens (Berk.) de Bary (Sacc. VII: 251) on leaves of Argemone mexicana L., Leg. Sinha, L. S. College, 17-2-60.
- 4. Peronospora viciae (Berk.) de Bary. (Sacc. VII: 245) on leaves of Pisum Sativum L., Leg. S. S. Pd., L. S. College, 3-2-60.
- 5. Peronospora parasitica (Pers.) de Bary (Sacc. VII: 249) on leaves of Raphanus sativus L., Leg. S. S. Pd., Jail Road, 12-10-60.
- 6. Phytophthora parasitica Dastur (Sacc. XXIV: 37) on leaves of Ricinus communis L. and on leaves of Piper betle L., Leg. B. D. Sinha, Kalambag Road, 18-3-60 and 15-11-60 respectively.
- 7. Phytophthora colocasiae Racib. emend Thomas and Ramakr. T. S. (Proc. Indian Acad. Sci. Sec. B; 27: 55) on leaves of Colocasia esculentum (L.) Schott., Leg. B. D. Sinha, Khabra Road, 10-8-60.
- 8. Protomyces macrosporous Unger (Sacc. VII: 319) on stem and fruits of Goriandrum sativum L., Leg. Sinha, Khabra Road, 15-3-61.
- 9. Synchytrium collapsum Syd. (Sacc. XXI: 839) on leaves of Clerodendron infortunatum Gaertn., Leg. S. S. Pd. Mithanpura, 6-3-60.
- 10. Synchytrium rytzi Syd. (Sacc. XXI: 840) on leaves of Leucas aspera Spreng., Leg. S. S. Pd., Mithanpura, 5-8-61.

#### **ASCOMYCETES**

- 11. Erysiphe polygoni DC. (Sacc. I: 19 as E. martii) on leaves of Pisum sativum L., Leg. Sinha, L. S. College breeding ground, 15-2-60.
- 12. Erysiphe cichoracearum DC. (Sacc. I under several names) on leaves of Lagenaria vulgaris Ser., Leg. Sinha, Nayatola, 20-11-60.
- 13. Erysiphe graminis DC. (Sacc. I: 19) on leaves of Triticum vulgare Vill., Leg. S. S. Pd., Mithanpura, 16-3-61.
- 14. Phyllactinia corylea var. subspiralis Salm. (Imp. Coun. of Agr. Res. India Sci. Mono. 1, XVIII: 35) on leaves of Dalbergia sisso Roxb., Leg. S. S. Pd., Mithanpura, 25-12-60.
- 15. Taphrina maculans Butl. (Sacc. XXIV: 1303) on leaves of Curcuma longa L., Leg. Sinha, Maripur, 8-12-60.
- Erysiphe sps. on leaves of Croton sparsiflorus. Morung., Leg. B. D. Sinha,
   L. S. College, 3-!-60 (A new host record).

#### **BASIDIOMYCETES**

- 17. Entyloma oryzae Syd. (Sacc. XXIII: 625) on the leaves and leaf sheaths of Oryza sativa L., Leg. S. S. Pd., University area, 12-10-60.
- 18. Sphacelotheca sorghi (Lk) Clinton (J. Mycol., 8: 140) in the ovaries of Andropogon sorghum Brot., Leg. S. S. Pd., University area, 12-11-60.
- 19. Graphiolo applanata Syd. & Butl. (Sacc. XXI: 526) on leaves of Phoenix sylvestris Roxb., Leg. S. S. Pd., University area, 20-11-60.
- Neovossia horrida (Taka) Padwick & Azmatullah Khan (Mycol. pap., No. 10: p. 2, 1944) in the ovaries of Oryza sativa L., Leg. S. S. Pd., Mithanpura, 15-11-60.
- 21. Ustilago scitaminea Syd. (Ustilaginales of India-1954 C. M. I.-p. 36) in the inflorescence & culms of Saccharum officinarum L., Leg. Sinha, Mahamda, 10-1-60.
- 22. Ustilago cynodontis P. Henn. (Sacc. XIV: 416) in the inflorescence of Cynodon dactylon Pers., Leg. Sinha, Mahamda, 3-12-60.
- 23. Ustilago tritici (Pers.) Rostrup (Sacc. IX: 282) in the ovaries of Triticum vulgare Vill., Leg. Sinha, Mahamda, 15-3-60.
- 24. Ustilago avenae (Pers.) Rostrup (Sacc. 1X: 283) in the ovaries of Avena sativa L., Leg Sinha, Mahamda, 10-4-60.
- 25. Ustilago hordei (Pers.) Lagerh. (Sacc. IX: 263) on Hordeum vulgare L., Leg., B. D. Sinha, Sikandarpur, 10-2-60.
- 26. Ustilago kolleri Wille (Bot. Notiser, 1893, p. 10) in the ovaries of Avena sativa L., Leg., Sinha, Maripur, 10-1-50.
- 27. Ustilago nuda (Jensen) Rostrup (Sacc. IX: 283) on Hordeum vulgare L., Leg., Sinha, Sikandarpur, 10-2-61.
- 28. Uromyces appendiculatus (Pers.) Link. (Sacc. VII: 535; Syd. II: 120) on leaves of Dolichos lablab L., Leg. S. S. Pd., Jail Road, 10-8-61.
- 29. Puccinia butleri Syd. (Sacc. XXI: 651) on leaves and stalks of Launaea asplenifolia DC., Leg. S. S. Pd., L. S. College garden, 10-2-61.

- 30. Puccinia graminis Pers. var. tritici Erikss. & Henn. (Imp. coun. Agric. Res. India Sci. Monogr. No. 14, page 16) on leaves and clums of Triticum vulgare Vill., Leg. S. S. Pd., Zila School area, 9-3-60.
- 31. Puccinia glumarum (Schm.) Erikss & Henn. (Sacc. XVII: 380; Syd. I: 706) on leaves and culms of Triticum vulgare Vill., Leg. S. S. Pd., Mithanpura, 19-2-60.
- 32 Puccinia triticina Erikss. (Sacc. XVII: 376; Syd. I: 716) on leaves of Triticum vulgare Vill., Leg. Sinha, Khabra Road, 9-2-60.
- 33. Puccinia kuehnii (Krueg.) Butl. (Sacc. XXIII: 744; Syd. IV: 608) on Erianthus munja & Erianthus arundinaceum Retz., Leg. S. S. Pd., Ramdayalunagar, 24-2-60.
- 34. Puccinia maydis (Bereng.) (Sacc. VII: 237) on leaves of Zea mays L, Leg. S. S. Pd, Ramdayalunagar, 10-10-60.
- 35. Puccinia cynodontis (Lacroix) (Sacc. VII: 661; Syd. I: 748) on leaves of Cynodon dactylon Pers., Leg. S. S. Pd., L. S. College, 15-12-60.
- 26. Puccinia helianthi Schw. (Sacc. VII: 603) on leaves of Helianthus annus L., Leg. S. S. Pd., L. S. College, 12-3-60.
- 37. Melampsora lini (Pers.) Lev. (Sacc. VII: 588; Syd. III) on leaves and stems of Linum usitatissimum L., Leg. Sinha, University area, 15-3-60.

#### DEUTEROMYCETES

- 38. Alternaria solani (Ell. & Mart.) Jones & Grout (Sacc. IV: 530 as Macrosporium) on leaves of Solanum tuterosum L., Leg. Sinha, Bose garden, Muzaffarpur, 20-12-60.
- 39. Alternaria brassicae (Berk.) Sacc. (Sacc. IV: 546 as Macrosporium) on leaves of Brassica oleracea L., Leg. Sinha, Bose garden, Muzaffarpur, 18-2-61.
- 40. Cephalosporium sacchari Butl. (Sacc. XXV: 651) in culms of Saccharum officinarum L., Leg. Sinha, Mahamda, 10-1-60.
- 41. Colletotrichum falcatum Went. (Sacc. XI: 570) on leaf sheaths and culms of Saccharum officianarum L., Leg. Sinha, Mahamda, 10-1-60.
- 42. Gercospora indica Singh (Ind. J. Agirc. Sci., 4:358) on leaves of Cajanus indicus Spreng., Leg. S. S. Pd., University area, 12-12-60.
- 43. Gercospora ricinella Sacc. & Berl. (Sacc. IV: 456) on leaves of Ricinus communis L., Leg. S. S. Pd., L. S. Gollege, 12-12-60.
- 44. Cercospora solanacea Sacc. (Sacc. 1V: 449) on leaves of Solanum melongena L., Leg. S. S. Pd., L. S. College, 10-9-60.
- 45. Gercospora feuilleauboisii Sacc. (Sacc. IV: 449) on leaves of Solanum migrum L., Leg. S. S. Pd., L. S. College, 10-8-60.
- 46. Cercospora amorphophalli P. Henn. (Sacc. XVIII: 611) on leaves of Amorphophallus companulatus Blume., Leg. Sinha, 5-8-61.
- 47. Gercospora calotropidis Ellis & Everh. (Sacc. XVI: 1072) on leaves of Galotropis gigantia Br., Leg. Sinha, Punkhatoli, 5-8-61.

- 48. Gercospora zinniae Ell & Mart. (Jour. Mycol. 1:20) on leaves of Zinnia elegans Jacq., Leg. Sinha, Saraisayedali, 12-11-60.
- 49. Fusarium udum Butl. (Sacc. XXII: 1479) on roots of Cajanus indicus Spreng., Leg. S. S. Pd., Mithanpura, 10-1-60.
- 50. Piricularia oryzae Cav. (Sacc. X: 563) on Oryza sativa L., Leg. S. S. Pd., Zila School area, 18-9-60.
- 51. Helminthosporium oryzae Breda de Hann (Sacc. XXII: 1394) on leaves of Oryza sativa L., Leg. Sinha, Khabra Road, 18-9-60.
- 52. Helminthosporium sativum Pammel, King & Bakke (Report of the Agricultural Research Institute & College, Pusa by Mc Rae 23: 55, 1922) on Hordeum vut gare L., Leg. S. S. Pd., Mithanpura, 21-3-61.
- 53. Phyllosticta artocarpina (Syd. & Butl.) Syd. (Sacc. XXV: 56) on leaves of Artocarpus integrifolia L., Leg. Sinha, Punkhatoli, 5-9-61.
- 54. Gloeosporium psidii Delacr. (Sacc. XVIII: 451) on leaves of Psidium guayava L., Leg. Sinha, Amgola Road, 5-9-61.
- 55. Pestalotia pauciseta Sacc. (Sacc. XXV: 608) on leaves of Nephelium litchi Camb., Leg. S. S. Pd., Amgola Road, 5-9-61.
- 56. Pestalotia mangiferae P. Henn. (Mycologia, 34: 308-17) on leaves of Mangifera indica L., Leg. S. S. Pd., College garden, 5-9-61.

# STUDIES ON NEW GALL MIDGES VIII (ITONIDIDAE: CECIDOMYIIDAE: DIPTERA) FROM INDIA 1

By

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The present paper, for the first time, records two new species of the genus *Micromyia*, one from Mihijam (Bihar) and the other from Allahabad (Uttar Pradesh), previously described species being from New Delhi.

# Tribe Micromyini

- 1840. Micromyia, Rondani, Sopra Alc. Gen. Insect. Dipt., Men. Sec. Serv. Italy, p. 21.
- 1911. Micromyia, Felt, J. N. Y. ent. Soc., 19: 33.
- 1913. Micromyia, Kieffer, Bull. N. Y. St. Mus., 165: 162.
- 1937. Micromyia, Mani, Rec. Indian Mus., 39(3): 281.

# Micromyia Rondani

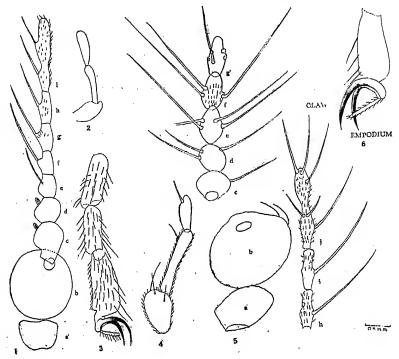
Micromyia Rondani is one of the very old genera, having been named in the year 1840 with M. lucorum Rondani as the type of the genus. Mani (1937) recorded this genus from the old world and described M. indica. This is the second record under this genus.

# Micromyia orientalis sp. nov.

Male: Body length 0.09 mm. palish brown; Head with three ocelli, eyes confluent above. Palpi (Fig. 2) triarticulate dark brown, sparsely setose, first segment narrow at the base and broad at apex, second segment cylindrical, a little less than twice the length of the first segment and four times as long as broad; third segment short, (shortest of all segments), wider sub-apically, length two-and-a-half times of its maximum thickness. Antenna dark-brown, to pale-brown, short a little more than one-fourth the length of the body, with eleven segments, ninth, tenth and eleventh segments fused to-gether, rest of the segments without distinct stems and each with a whorl of long setae in the middle, scape (Fig. 1a) dark-brown, nearly rectangular, wider than long, length a little less than half the maximum thickness; pedicel (Fig. 1b) enlarged, globose, a little more than twice the length of the first segment; third segment (Fig. 1c) short, two-third the length of the pedicel, wider sub-apically, length one-and-one-thirds the maximum thickness; fourth segment (Fig. 1d) shorter than the third, as long as the first segment and as long as broad; fifth segment (Fig. 1e) as long as the fourth segment but slightly narrower, length one and two-thirds the maximum thickness, sixth segment (Fig. 1f)

<sup>1.</sup> This work has been completed under the tenure of Govt. of India Junior Research Scholarship.

as long as the fifth segment but narrower than the latter, length two-and-a-half times its maximum thickness; seventh (Fig. 1g) a little longer than the sixth segment, cylindrical with bulging sides, length thrice the maximum thickness:

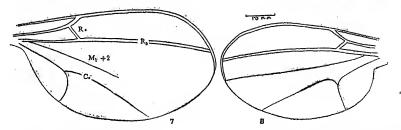


Text-figures 1-6 showing antennae, palpi and claws of Micromyia orientalis and M. championii.

1. Antenna: a. Scape; b. Pedicel; c. Third antennal (segment; d. Fourth antennal segment; e. Fifth antennal segment; f. Sixth antennal segment; g. Seventh antennal segment; h. Eighth antennal segment; t. Ninth, tenth and eleventh antennal segments; 2. Palpus of M. orientalis; 3. Hind leg and claw of M. orientalis; 4. Palpus of M. championii; 5. Antenna of M. championii: a. Scape; b. Pedicel; c. Third antennal segment; d. Fourth antennal segment; e. Fifth antennal segment; f. Sixth antennal segment; g. Seventh antennal segment; h. Eighth antennal segment; i. Ninth antennal segment; j. Tenth and eleventh antennal segments; 6. Hind claw of M. championii.

eighth segment (Fig. 1h) similar to the sixth segment, ninth, tenth and eleventh segments (Fig. 1i) confluent, as long as second segment, length six times the maximum thickness, terminal segment conical at the apex. **Thorax**: Mesonotum dark-brown, scutellum and post-scutellum light brown; halteres pale-yellow. Wing (Fig. 7) hyaline, twice as long as broad, vein  $R_1$  nearly six times the length of the vein  $R_5$ , vein  $R_5$  extending to the tip of the wing, costa cantinued beyond its union with vein  $R_5$ , vein  $M_{1-2}$  faint and upto half the length of the wing, vein Cu forked. Legs not very long, light brown, covered with scale-like setae, metatarsus long, longer than the second tarsal segment and twice the length of the third and terminal segment, third segment equal to the terminal tarsal segment; Claw (Fig. 3) simple evenly bent, empodium narrow and shorter than the claw. Genitalia (Fig. 9) dark-brown, densely setose, basal clasp segment stout, long, twice as long as broad, terminal clasp segment three-fifth the length of the basal clasp segment and thrice as long as broad, densely setose, setae long, sub apically armed with a short, sharp

spine on the inner side of the segment, dorsal plate longer than the ventral plate, narrowly incised in the middle, lobes triangular, emarginate, inflected ventrally, ventral plate much narrower than the dorsal plate, slightly notched in the middle, lobes rounded apically, style shorter than the ventral plate, rounded apically.



Text figure 7. Wing of M. orientalis.

Text-figure 8. Wing of M. championii.

# Holotype:

One male dissected and mounted on slide, labelled, 'on grass," P. G. coll, Mihijam, Bihar, dated the 19th Jan. 1960.

### Paratype:

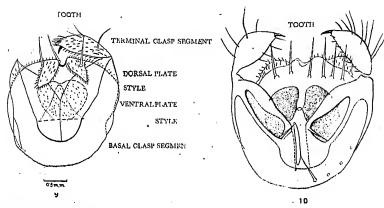
Many males in spirit and six mounted on slides and labelled as holotype. Slides retained in the collection of the author for the time being.

# Micromyia championii, sp. nov.

MALE: Body light-brown, length 1.6 mm. Head dark-brown with three ocelli. Palpus (Fig. 4) triarticulate, light brown, densely setose, first segment broad apically, wider than all segments, one and two-thirds as long as thick at apex; second segment cylindrical slightly broad apically, narrow in the middle, one and a half times longer than the first segment and five times as long as thick in the middle, third segment shortest of all segments, nearly half the length of second, broad medially, densely setose, a little more than twice its thickness, conical apically Antenna dark brown, shorter than body; scape (Fig. 5a) dark brown, nearly rectangular, slightly wider than long; pedicel (Fig. 5b) enlarged, dark brown, darker than scape, globose; third segment (Fig. 5c) short, nearly globose, lighter in colour, less than one-third the length of the second segment; fourth segment (Fig. 5d) shorter than the third segment, slightly broader than long; fifth segment (Fig. 5e) as long as third segment, slightly narrower at the middle and sub-cylindrical; sixth segment (Fig. 5f) slightly longer and narrower than the fifth segment, cylindrical, broad medially, twice as long as thick; eighth segment (Fig. 5h) as long as, but slightly narrower than the seventh segment, two and two thirds as long as thick; ninth segment (Fig. 5i) slightly longer than the eighth segment and thrice as long as thick; tenth and eleventh segments (Fig. 5j) fused together, as long as second segment and six times as long as thick, terminal segment rounded apically. Thorax: Mesonotum dark-brown, scutellum light brown. Wing (Fig. 8) hyaline, nearly twice as long as broad, vein R<sub>1</sub> nearly six times the length of vein R<sub>3</sub>, vein R<sub>5</sub> extending to the tip of wing, costa continued beyond its union with vein R5 for a short distance, vein M1-2 complete and distinct, vein Gu forked. Legs dark brown,

<sup>1.</sup> Named after the locality.

densely covered with scale-like setae, with five segments, metatarsus longer than the second and third tarsal segments combined, twice the length of second segment, third segment more than one third the length of metatarsus, terminal segment equal to the third segment; Claw (Fig. 6) dark brown, strongly bent, empodium half the length of the claw; Genitalia (Fig. 10) dark brown to light brown, basal clasp segment cylindrical, heavily chitinised basally and peripherally, less chitinised in the middle, nearly twice as long as broad, terminal clasp segment nearly half the length the basal clasp segment, oval, thrice as long as broad, densely setose, with an apically small, sharp, straight spine at the tip, dorsal plate much longer and broader than the ventral plate and beset with a row of long, stout setae subapically, sligthly incised in the middle, lobes rounded, margin strongly emarginate, densely setose, ventral plate deeply and narrowly incised in the middle, lobes rounded as long as broad, style shorter than the ventral plate, broadly rounded apically, five times as long as broad.



Text-figure 9. Genitalia of M. orientalis.

Text figure 10. Genitalia of M. championii.

#### Holotype:

One male dissected and mounted on slide and labelled, "at light, H. S. Siddiqi coll. Champion Garage, Civil lines, Allahabad, Dated 18 10-61".

### Paratype:

Two males mounted on slides and a few in spirit, labelled as holotype.

# Key to species

1. Second palpal segment shorter than the third palpal segment, antenna with ten segments, ninth and tenth segments fused together, segments with distinct stems; claw sickle shaped...,M. indica Manj

Second palpal segment longer than the third segment; antenna with eleven segments without distinct stems, vein M<sub>1-2</sub> complete  2. Second palpal segment twice the length of the first segment; antenna with ninth, tenth, and eleventh segments fused together, vein  $M_{1-2}$  incomplete, empodium slightly shorter than the claw; dorsal plate slightly incised, lobes triangularly emarginate, ventral plate slightly notched in the 

Second palpal segment two-and-a-half times the first segment; antenna with tenth and eleventh segments fused together, vein  $M_{1-2}$ complete; empodium half the length of the claw, dorsal plate slightly incised, lobes rounded, strongly emarginate, ventral plate deeply and narrowly incised in the middle...M. championii sp.nov.

#### DISCUSSION

# Palpus:

In M. orientalis and M. championii third palpal segment is shortest of all palpal segments, whereas in M. indica the third palpal segment is longer than the first but shorter than the second segment. Further the second palpal segment in M. orientalis (Fig. 2) is less than twice the length of the first segment and four times as long as thick while in M. championii (Fig. 4) the second segment is one-and-a-half times longer than first and five times as long as thick.

In M. orientalis the antenna is less than one-fourth the length of the body, whereas in M. indica it is less than one-fifth the length of the body. In M. indica the antenna consists of ten segments and ninth and eleventh segments are fused together, while in the case of M. orientalis (Fig. 1) and M. championii (Fig. 5) the antenna is with eleven segments, but in M. orientalis ninth, tenth and eleventh segments are confluent, whereas in M. championii only tenth and eleventh segments are fused together. Seventh and eighth segments are with distinct stems in the case of M. indica, whereas in M. orientalis and M. championii the antennal segments are without distinct stems, but segments second to fourth are furnished with sensory setae which are not mentioned in Mani's description.

#### Wing:

In case of M. orientalis (Fig. 7) vein  $M_{1-2}$  is faint and it reaches upto half the length of the wing whereas, in M. championii (Fig. 8) vein  $M_{1-2}$  is complete. In the case of M. indica, however, Mani has not given any description of the wing.

#### Claw:

Empodium is shorter than the claw in the case of both M. orientalis and M. championii, (Fig. 3 to 6) but Mani has not mentioned any thing about the empodium and its proportion with claw.

#### Genitalia :

Basal clasp segment of M. orientalis (Fig. 9) is stout, long and twice as long as broad, whereas in M. championii (Fig. 10) it is cylindrical, nearly twice as long as broad and is heavily chitinised at the base, periphery and is less chitinised in the middle. In the case of M. indica basal clasp segment (Figure not given) is stout and short. Terminal clasp segment in the case of *M. orientalis* is three-fifth the length of the basal clasp segment, whereas in *M. championii* it is half the length of the basal clasp segment, oval but in *M. indica* terminal clasp segment is sub-fusiform and is about one-third the length of the basal clasp segment. Terminal clasp segment is armed apically with a short, straight sharp spine which is stiutated sub-apically on the inner side of the tip in the case of *M. orientalis* but in *M: championii* the spine is at the tip. On the other hand in the case of *M. indica* it is armed apically with a short and slightly curved sharp spine.

Dorsal plate in the case of M. orientalis is broad and longer than the ventral plate but shorter than the basal clasp segment. It is narrowly incised in the middle having triangular lobes which are emarginate and reflected ventrally, whereas in M. championii dorsal plate is much broader and longer than the ventral plate. It is also longer than the basal clasp segment. It bears a row of long and stout setae on the sub-apically portion, in the middle it is slightly incised. In this case lobes are rounded with strongly emarginate margins. But in the case of M indica the dorsal plate is longer than the basal clasp segment and it is bilobed apically and emarginate.

Ventral plate is much narrower than the dorsal plate and is notched in the middle with rounded apical lobes in *M. orientalis* while in *M. championii* it is deeply and narrowly incised in the middle with broadly rounded lobes. Nothing is mentioned about ventral plate in the case of *M. indica* by Mani.

#### Style:

In M. orientalis the style is shorter than the ventral plate and is rounded at the apex while in M. championii it is broadly rounded apically and is five times the maximum thickness No description of the style is avail in the case of M. indica.

# ACKNOWLEDGMENT

The author is indebted to Prof. M. D. L. Srivastava, Head of the Zoology Department, University of Allahabad, for providing facilities for her work. She expresses her deep sense of gratitude to Dr. S. N. Prasad for supervision and guidance. She is also grateful to Dr. S. N. Rao for encouragement and help.

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STUDIES OF INTRASPECIFIC VARIATIONS IN TREMIORCHIS RANARUM MEHRA ET NEGI, 1926 (PLAGIORCHIDAE LÜHE, EMEND WARD, 1917: TREMATODA) AND DISCUSSION ON CAUSES AND EFFECTS OF INTRASPECIFIC VARIATIONS

B'

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#### INTRODUCTION

Tremiorchis ranarum Mehra et Negi, 1926 is a plagiorchid trematode which parasitizes amphibians, especially Rana tigrina. T. ranarum is of unusual interest in several respects. In structure it is mainly of a plagiorchid nature, and yet it shows considerable divergence from this line of evolution. The shortening of the intestinal caeca and a limited extension and size of vitellaria are characters typical of the dicrocoelids. This affinity is further supported by the fact that these closely related trematodes occur in related hosts. This view was also stressed by Mehra (1931). He concluded that Brachycoelium (Dujardin, 1845: sub genus stiles and Hassal, 1898) appeared to have evolved from Tremiorchis Mehra et Negi, 1926, and he even kept the subfamily Brachycoelinae Looss, 1899 under the fam. Plagiorchidae. At present Brachycoelinae is rightly considered as a subfamily of family Dicrocoelidae. And this author feels justified in having put the genus Tremiorchis in a separate subfamily Tremiorchinae n. subfamily (the paper with Parasitology) under the family Plagiorchidae.

The information presented in this paper records further observations on T. ranarum. About 150 specimens obtained from over 400 frogs, were studied in life as well as in permanent slides, and this study has indicated interesting morphological variations. A short discussion of the probable causes of such variations, with a discussion of the possible evolutionary significance of these intraspecific variations has been given here, in the light of the present day knowledge.

#### VARIATIONS

The following interesting variations have been noticed in the specimens of *Tremiorchis ranarum* studied by the author, and these have been illustrated by a few diagramatic sketches.

#### Size:

The variance in size though of not much importance is quite marked here. Mehra et Negi (1920) have reported  $4-5.6 \times 1.18$  mm. and in the present study the dimensions of the worm were not found more than  $3 \times 1.7$  mm.

<sup>\*</sup>Now re-named as Government Science College.

# EXPLANATION OF PLATES

Only Fig.B Plate II has been prepared by camera lucida, the rest of the figures are diagramatic, true representations of the slides.

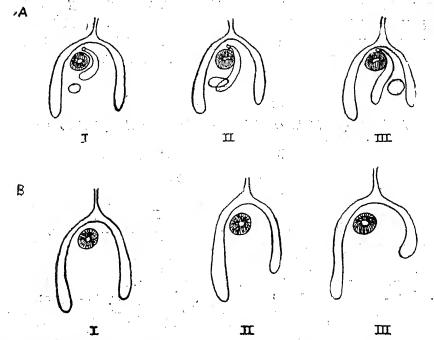


Plate I. Figs. in A (1-3) show variations of cirrus sac, figs. in B show a symmetrical shortening of the caeca.

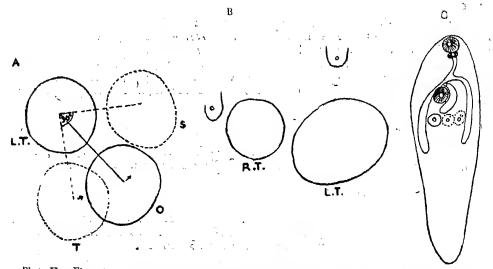


Plate II. Figs. in A indicate rotation of the right testis, and its various observed positions.

Fig. B shows maximum variation in size of testes. Fig. C indicates positional variations of ovary.

#### Suckers:

Mehra and Negi (1926) have shown a ratio of 3:4 in the suckers and the acetabulum has been reported as the bigger Whereas, in the present study an acetabulum smaller than the oral sucker has also been noticed, not to speak of the subequal suckers

#### Intestinal caeca:

The left as well as right caeca have shown independent shortening of a rather smaller degree in most cases, but in one instance, illustrated here one caecum has shown a very marked shortening.

Similarly, indeterminate and haphazard variations have been found in genitalia also quite often, and they are all quite meaningful in the author's opinion:

Testis:

In a normal form of Tremiorchis ranarum the right testis has been reported to be slightly posterior to the left testis, which in its turn lies near the caecal ending of that side. The size of the right testis has been noticed to be slightly larger also. The present author has seen some spectacular variations in the position and size of the testis. Three features have been observed to show big variations in Tremiorchis ranarum. Firstly, the study of various specimens has shown that the right testis has a tendency to rotate through at least 90° or more (as shown). Starting from the tandem position, all the intermediate positions exist. Even the side-by-side position, so emphatically denied for this genus by Mehra and Negi (1926), exists, and the right testis has gone a little higher as well. The more commonly observed position, no doubt, is the oblique one.

The size is also not constantly larger in the right testis. The right testis may even be smaller or equal in size, more or less. In one strange variation the right testis has become much smaller than that of the left one. The ratio in this particular case is 1:2.

# Ovary:

Ovary also shows positional changes in Tremiorchis ranarum. It may be higher or lower in the body, or displaced towards one or the other side.

#### Cirrus Sac:

Has shown variations of shape and size in this species. Although Varma (1930) has called it more dorsal than lateral to the acetabulum, this point is not acceptable, at least in *T. ranarum*. Mehra's and Negi's (1926) lateral and left to the acetabulum position of cirrus sac is quite correct.

Here, cirrus sac has been shown to become comparatively much larger in its posterior extent, sometime taking the shape of a sign of interrogation (?). In some cases, the enclosed ducts have shown lengthening, and the ductus ejaculatorius instead of being, the usual straight, becomes somewhat looped in its second half. The shape and extent of the cirrus sac has been given a taxonomic importance in plagiorchids by various authors, hence the importance of this variation.

#### Vitelline gland:

This too has shown considerable variations of size, extent, arrangement and position. More often than not, it has been observed to possess a typical grouping of 3 continuous clusters on each side, rather than 4 or 5 in an astral fashion as mentioned by Mehra and Negi (1926). Another extraordinary variation which has been observed is the dwindling of the number of follicles on one or the other side. In one particular case they had become very few only on one side.

Having studied these variation, one would not fail to realise the controversial situations which might arise due to such haphazard and indeterminate variations for the taxonomist. At the same time it should not be difficult to recognise the true and stable specific characters of a species which might be under study. These variations are discontinuous, and of an indeterminate nature, and generally speaking, in the morphological picture of a particular specimen there should not be any mistaking, in case such variations appear.

However, one is face to face with the problem of the probable causes which work these morphological changes in this particular species, and for that matter, of course, in the parasitic flat-worms and animal kingdom in general. The author is of the opinion that variations, when they are of an inderterminate and haphazard type are due to "genetic plasticity." According to de Beer (Embryos and Ancestors, 1951), "Genetic plasticity is the ability of individuals of a species to show a high degree of variance, and this condition obtains when the number of individuals carrying genes in the heterozygous condition, and the number of those genes is large. In such species the possibilities of recombination of genes are numerous, and there is a reserve of recessive genes which may come into play, in one way or another, in the new condition which recombinations and permutations provide". Various other types of plasticity exist but here the abovementioned type ought to exist along with its helper and complement the environmental or ecological plasticity.

It is well-known, and a very extraordinary fact that trematode parasites, and more particularly the parasites of amphibians and other cold-blooded animals (in the case of amphibians the factor of hibernation of the host is considered by the author as a very pertinent ecological factor) have to succeed through a very notoriously hard struggle for existence, hence genetic plasticity comes into full play. The highly specialised body of the internal parasites, which lies bare to the environments (even more than the tiny insects), not only shows haphazard variation, as discussed in the case of Tremiorchis ranarum, but ultimately, due to certain chance combinations of genes, mutations occur. Hence, possibly, genetic plasticity has very powerfully contributed to evolutionary variance in helminthic parasites. Their successive specialization and the genetic plasticity must have made an overwhelming contribution to the success of helminthic parasites as a group.

Stunkard (1957) in his thoughtful paper, Intraspecific variations in Parasitic Flatworms, has certainly made the situation about parasitic flatworms much clearer. And the idea of genetic plasticity becomes more convincing when we consider Stunkard's remarks in this paper, "and the 'gene', I venture to suspect, is merely a stereoisometric configuration of constituents in the protoplasm, which determines the kind and rate of reactions that take place in it. These reactions, predetermined by the substrate and mediated by enzymes, determine the endproducts of seccessive reactions and direct the course of morphogenesis." Further, Stunkard has summed up in this paper, "It is abundantly clear that flatworm parasites are able to acquire new hosts, and that they change hosts with differing ecological situations. It is equally clear that development in different host-species and under different physiological conditions of the individual host may profoundly alter the parasites."

Another very appealing and enlightening reference is that of La Rue (1951) in his paper, Host relations of trematodes. Only a couple of quotations are put in here:

"In general, it can be stated that species of Digenea are well adapted to the various conditions imposed upon them during the various stages of the life-cycle, assuming that events follow their normal course of media and hosts." "Each species seems to play the game of parasitic life within the broad rules laid down by, and for, its family; but it appears to have developed within this code its own special rules and regulations, its own deviations from what we poor humans assume to be normal and regular for the family." These remarks definitely give us the natural corollary that there has to be the maximum amount of structural, ecological and genetic plasticity in parasitic flatworms in general, and Digenea in particular, so that they be successful in their uniquely stubborn struggle for existence, and evolution. And no wonder, endowed with such gifts, the Digenea show the peculiar intraspecific variations, and the extraordinary evolutionary set up, and their great successfulness in the animal kingdom.

Many other authors, e.g., Dawes (1946), de Beer (1951), Dobzhansky (1951), Huxley (1942), Huxley and others (1940) Mayr, Linsley and Usinger (1953) who have made the bulk of ideas about this controversial and elusive subject (they have been duly placed in the bibliography of this paper), besides, many more authors have been studied, and all this has enabled the author to come to suggestions given in this paper.

Only when the divergence is determinate and conspicuous enough and can be brought to the touchstone of genetics, it definitely speaks of a new taxon. On the contrary, if the variations be indeterminate (as in the above observations) no such value can be attached to them, although such variations too have their own definite value, as discussed above. It is significant to note that flatworm families are freely sprinkled with species suffering from variations of this nature, although, there is also no dearth of such species which have attanied a phase of considerable stability (obviously a temporary one). Indeed, the author feels that these variations, or rather the plasticity is of great significance for the evolution of flatworms in particular and all the other creatures at large. It is only due to such qualities of the parasitic flatworms that we find the remarkable abundance of the species showing either convergent or divergent evolution, which obviously have given rise to difficult taxonomic puzzles.

Moreover, the author feels that there is an evolutionary tendency in *T. ranarum* as far as the rotation of the right testis is concerned. Whereas, normally, in *T. ranarum*, it does not exceed about 90°, the rotation has become almost 180°, and when it becomes 180° a permanent change in the normal species seems to have occurred. And along with the slight lengthening of the caeca, the feature of the right testis becoming intracaecal confers the status of a new species *T. varanum* Verma, 1930, which was described by him from the two species of a new and reptilian host *Varanus*.

Having ventured to make some observations about the evolutionary and other values of the genetic plasticity in parasitic flatworms, and in other animals as well, the author wishes to conclude his paper with a few necessary remarks about the unit of taxonomy, and obviously it is just a corroboration to the opinions of the various eminent scientists alluded to above. "Species" has been rightly considered the fundamental unit of taxonomy, and from the scientific point of view it is perfectly logical too. Similarly, the larger unit of genus is equally a self asserting one. But there seems little justification in still upholding by many taxonomists the erroneous, unscientific and pseudotaxonomic concept of subspecies and subgenera, and Huxley, Dobzhansky and various other workers have rightly pleaded against the use of these pseudotaxa. Doing away with them definitely helps in the clearing

of our ideas about speciation, and intraspecific variations, and hence about taxonomy as a whole.

#### ACKNOWLEDGMENTS

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# LIFE HISTROY AND CONTROL OF CHILO ZONELLUS SWINHOE AN IMPORTANT PEST OF JUAR AND MAIZE

B

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#### INTRODUCTION

Juar and maize are most important grain crops of Kharif season and occupy extensive area in Uttar Pradesh. These crops are attacked by a number of insect pests of which maize and juar borer, Chilo zonellus Swinhoe, (Family Pyralidae Order Lepidoptera) is an important one and regularly causes damage to them all over the State of Uttar Pradesh. The pest has also been observed attacking Bajra, sugarcane and paddy. In India this pest has been reported from all over the country.

#### LIFE HISTORY

#### Adult:

The straw coloured, nocturnal moths are found during day resting among leaves, on stems and under clods in the fields. They are attracted to incandescent light in the night. In laboratory the average longevity of male and female moths is observed to be 3 and 4 days repectively. The copulation period ranges from 1 to 6 hours while its average comes to 4 hours.

# Oviposition period:

The oviposition period in this insect is observed to vary from 1 to 5 days depending on the existing temperature and humidity.

#### Eggs:

Females lay oval, dull creamy eggs in clusters on leaves and generally near the mid-ribs. Average fecundity per female is calculated to be 226 eggs. A single female in her life time may lay as many as 722 eggs.

#### Incubation period:

During the months of August, September and October the eggs hatch after 3 to 5 days. The eggs usually hatch in 4 days when average temperature and humidity during incubation period is 83.5°F and 78.4%, respectively.

#### Larva

The larva, after hatching out of the egg enters the stem and feeds on internal tissue. There may be one or more caterpillars present inside a single infested stem. The larva generally moults 5 times and rarely 7 times to become fully mature. The larval stage lasts from 18 to 42 days during different months of activity of this pest as is given in the Table I.

TABLE I
Showing the total larval period and period of various instars of the Juar stem borer, Chilo zonellus Swinhoe at Kanpur

Sl. No.	Period of 1st insstar in days	Period of 2nd instar in days	Period of 3rd instar in days	Period of 4th instar in days	Period of 5th instar in days	Period of 6th instar in days	Period of 7th instar in days	Period o. 8th instar in days	f Total larval Period in days
1.	1	4	3	2	5	9	-	Property	24
2.	1	5	2	3	6	10	<b>,</b>	polite	27
3.	1	4	2	2	3	4	7		23
4.	1	4	3	4	2	4		-	18
<b>5.</b>	2	3	4	5	3	10	-	-	27
6.	2	3	4	4	4	7			24
7.	4	3	4	1	2	3	3	6	26
8.	2	3	2	4	3	9		-	23
9.	3	3	2	3	3	8	-	-	2 <b>2</b>
10.	<b>4</b> ·	3	2	3	2	8		i=on	22
11.	4	3	1	3	3	9 .		Printe.	23
12.	5	. 1	1	4	3	7		_	21
13.	3	2	2	2	5	13	-	_	27
14.	3	4	4	3	10	12	prim	andra .	36
15.	4	5	5	.7	2	10		_	33
16.	5	2	3	4	5	12	11	-	42

The above tabe shows that the larval period of the Juar stem borer varies from 18 to 42 days during the different months of activity (July to November) of this pest; the larvae of this pest moult 5 to 7 times to become fully mature, when they pupate. Trehan (1949) has reported that normally there are five moults but the overwintering larvae showed an extra moult.

#### Pupa:

The fully mature caterpillar pupates inside the stem. The pupal stage is completed in 5 to 8 days. Emergence of adult from pupa generally takes place in the morning.

### Hibernation:

The pest hibernates in larval stage. Hibernation may start from end of October and extend till end of June. The caterpillars hibernate in stubbles, stems and sometimes in cobs. The hibernating caterpillars pupate at the earliest by the middle of March and latest by the last week of June.

#### CONTROL MEASURES

With a view to evolve a suitable method for its control a field insecticidal trial was conducted in *Kharif* season 1959 at Kanpur. In this experiment a simple randomised block design with four replications was adopted, the treat-

ments included in the trial being spraying with 0.25% Endrin + 1% ovicide, 0.25% Endrin alone, 0.175% Diazinon + 0.25% DDT, W. P., 0.1% Diazinon and 0.1% Lindane + 0.25% DDT, W. P. each @ 80 gallons per acre. The effects of these different sprays were studied with respect to the percentage of Juar plants affected by stem borer. The data was statistically analysed and the analysis of variance showed that variation due to treatments is significant. (Table II) The summary of results, giving the mean-percentage of affected plants is presented in Table III from which it would be seen that only two treatments viz. spraying with 0.25% Endrin + 1% ovicide and spraying with 0.25% Endrin alone gave significant result when compared with control

TABLE II

Analysis of variance of affected plants

Variation due	D. F.	S. S.	M. S.	F.
Replications	3	34.7143	11.5714	1.7601
Treatments	5	130.8036	26.1607	3.9793*
Error	19	· 124·9107	6.5742	
Total	27	290:4286		

<sup>\*</sup> Denotes significance at the 5% level probability.

TABLE III
Summary of results

Sr. No.	Treatments	Mean number affected Plants per 50 plants
1.	Spraying with 0.25% Endrin + 1% ovicide	
	@ 80 gallons per acre.	<b>ઠ•</b> 00
2.	Spraying with 2 lbs. of actual Endrin @ 80	
	gallons per acre.	9.00
3.	Spraying with 0.075 % Diazinon + 0.25%	
	DDT W. P. @ 80 gallons per acre.	11.00
4.	Spraying with 0.1% Diazinon @ 80 gallons	
-	per acre.	11.25
5.	Spraying with 0.1% Lindene + 0.25% DDT	
	W. P. @ 80 gallons per acre.	12.00
6.	Control	14.13
Name of Street, or other Designation of Street, or other Desig	General mean	11.36
	Critical difference at 5% Tevel	-

	Critical difference at 5% Level	
(a)	Between spraying treatments	3.79
(a) (b)	For individual syraying treatments	
• •	versus control	3.29

From the above results it is concluded that spraying the crop with 0.25% Endrin + 1% ovicide @ 80 gallons per acre proved to be the most effective of all the treatments and gave significant results (high control of pest) when compared with Control (No treatment).

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